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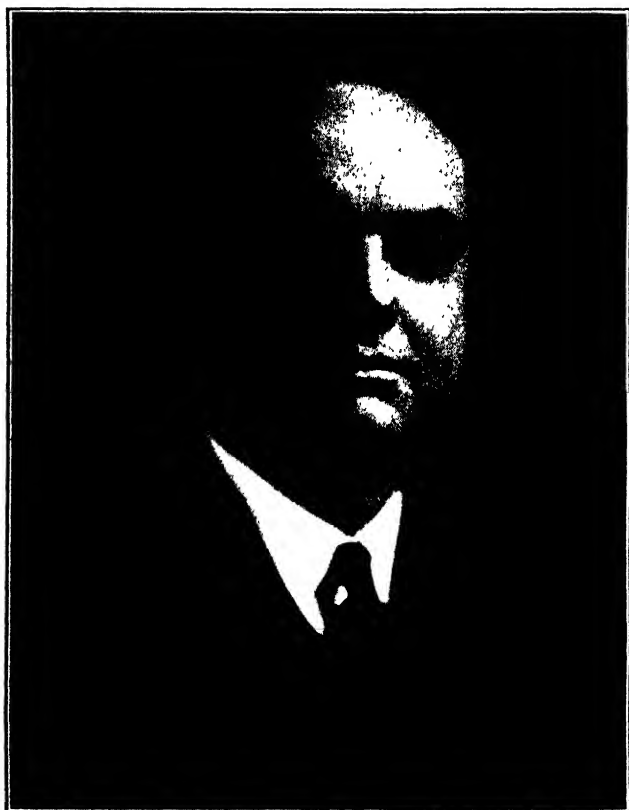
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REUBEN WILFRED BALCOM, 1877-1920.

REUBEN WILFRED BALCOM

Long before life's work was done the journey of R. W. Balcom came to a sudden end. After a full day at work, October 17, 1929, at the close of which he appeared especially happy in the knowledge of more than usual accomplishment, and while apparently in perfect health, he was stricken at his home shortly before the evening meal. Death followed four hours later at the Georgetown University Hospital in Washington, D. C.

Born in Morden, Kings County, Nova Scotia, November 18, 1877, he spent his boyhood with his grandparents in this picturesque fishing village on the Bay of Fundy, where life was full of the adventures of the open North Country. Perhaps the rugged land with its long, rigorous winters instilled something of its physical features into his character to make the man self-contained—not self-centered—resolute, isolated from casual contact, reserved for the few.

At the age of twelve he joined his mother at Framingham, Massachusetts, where she had made her home at the death of his father when the boy was four years of age. Those who knew his seriousness of purpose can easily picture this resolute lad doing chores and work of a varied character such as is offered in a small town so as to be of financial assistance. His love of the truth and devotion to honesty, early manifested, led his teacher in chemistry at Framingham to say that he would rather trust Wilfred than the Bank of England.

After three years of high school the young man entered the Massachusetts Institute of Technology, graduating in 1900 with the degree of B. S. While at "Tech" he became a member of the Technology Christian Association. His affiliation with this organization, as with the Alpha Lodge of Masons at Framingham, was kept throughout the years. The two years following graduation he served as Assistant Analytical Chemist at the Institute, and he was then awarded the Austin fellowship for foreign study. The three years in Europe were devoted to study at the Universities of Leipsic, Breslau and Heidelberg. It is not surprising that with his fetish for accuracy, thoroughness, and close application to details he received from Heidelberg, in 1905, the degree of Doctor of Philosophy, *magna cum laude*.

The next two years were spent as instructor in General and Physical Chemistry and in Analytical Chemistry at the University of Michigan. While at the University he was honored with membership in Sigma Xi. In 1907 he entered the Bureau of Chemistry of the U. S. Department of Agriculture in the work newly organized by reason of the passage of the Federal food and drugs act. After extensive experience in Washington and several field stations he was transferred, in 1909, to Nashville to take charge of the branch laboratory.

Before returning to Washington in 1914, when the Nashville Laboratory was discontinued, Dr. Balcom's keen interest in the advancement of his profession led him to a charter membership in the Nashville section of the American Chemical Society. He served the section as chairman from 1912 to 1914. In 1910 he was made a fellow of the American Association for the Advancement of Science. At Washington he became first assistant

to Dr. A. L. Winton in the establishment and operation of the Food Investigation Laboratory of the Bureau of Chemistry, a laboratory devoted to research. Promotion to the chiefship of the laboratory followed in 1920.

A desire to serve in activities leading toward the better enforcement of the law made him an active, ardent worker on the resulting problems, and a number of contributions to the scientific literature on the chemistry of foods resulted from his labors. Dr. Balcom always held the facts of any issue clearly before him and strove to see the right prevail. Such characteristics, together with his judicial temperament, made him an exceptional witness for the Government in many cases arising under the food and drugs act.

From 1922, when the Food Investigation and Food Control Laboratories were combined, until July 1, 1927, when the Food, Drug and Insecticide Administration was organized, Dr. Balcom was engaged in administrative editorial work. His methodical manner of doing things fitted him splendidly for this position, wherein he gathered technical information, especially that material in the Bureau not readily accessible, and from it prepared compilations that are clear and concise, and valuable alike to regulatory and research workers in the field of agricultural chemistry.

Although he contributed to the work of the Association of Official Agricultural Chemists from his first opportunity and served also as a referee, it was in the years from 1921 to 1929 that he gave unstintingly of his time and energies that the Association might succeed, prosper and progress. Following Dr. Alsberg's resignation in 1921 he served as Secretary-Treasurer from June until October. In 1921 he became chairman of the Board of Editors at a critical time in the life of the Journal of the Association. It was owing to his untiring patience, breadth of vision and adherence to high ideals that the Journal, beset with the difficulties common to all newly created, striving publications, became a fixed contribution to the field of the world's scientific literature. He held this position until 1927, when the press of other duties forced him to give it over to other hands. Following the death of Mr. Doolittle in 1926, the duties of the chairman of the Committee on Editing Methods of Analysis were also performed by Dr. Balcom until 1929.

With the establishment of the enlarged Food Control Laboratory in the newly created Food, Drug and Insecticide Administration, Dr. Balcom became Chemist in charge of the Laboratory. Scientific studies and researches in chemistry and bacteriology and investigations on the technology of foods which are conducted on problems arising from the regulatory activities of the Administration make this an important staff laboratory. A large personnel of scientific workers and assistants were proud to be associated with Dr. Balcom in the work and were inspired by his understanding sympathy and justice, and his regard for the rights of his fellow man. Concurrent with this last position he was also a member of the Joint Committee on Food Definitions and Standards.

In Nashville, on December 14, 1911, he was married to Nanita Collier MacDonell, a graduate of Vanderbilt University. To them were born three children, Margaret MacDonell, Harriet Webster, and Robert Wilfred. These members of his family survive him.

Owing to an innate modesty and a reluctance to talk about himself, few except his closest friends knew of Dr. Balcom's passion for music. It was the music that expresses life's deepest emotions, the Opera, that was his

delight, the Ring music of Wagner being favored. A study of the stars also offered a diversion, and it was his custom on clear nights to spend the fifteen minutes before bedtime on the front porch observing them. The opportunities of his boyhood life in the great outdoors led, no doubt, to his love for such adventure as the open places afforded. During his years in Europe summer recesses were spent in tramping with fellow students through the Schwarzwald and the forests of the Harz mountains, and in the mountain and lake country of Switzerland and Italy. The enjoyment of such physical activity was continued in all the localities in which he lived in this country and found its last expression in Sunday afternoon walks with his children in the woods about his suburban home. Such walks afforded an opportunity for instructing the youngsters on the ways of trees and birds. Athletic competition through tennis, golf and bowling also served as an outlet for his abundant energy. The care of a large vegetable garden, which he tended with mathematical precision, an expression of the skill of the scientist, later succeeded the more strenuous exercise. He spent many happy hours in genealogical research preparing for publication notes on the Balcom family.

To those who were fortunate enough to be associated with Dr. Balcom there remains a growing appreciation of his sterling character. He decided all things on fact and on merit uninfluenced by sentiment. One was always impressed by his integrity, frankness and loyalty—loyalty to his friends, to his work, and above all loyalty to duty. Dr. Balcom's whole character was exemplified in the three simple words of his book plate, "Be a man"; and there lived, there lives, no truer, finer, manlier man.

HENRY A. LEPPER.

EDITORIALS.

EDITORIAL POLICY.

With this number of *The Journal* the Board of Editors inaugurates a new policy in establishing an editorial column or section. It is proposed to present an editorial or series of editorials on current scientific work and on the activities of the Association of Official Agricultural Chemists. Related subjects in the agricultural chemical field and in regulatory work of fertilizers, insecticides, foods, drugs, and feeding stuffs will also be covered.

As is well known, the advances now being made in the field of applied chemistry are numerous, and it is with the idea of focusing attention on some of the most important of these advances that these editorials are presented.

The writers of the editorials will be chosen from the association membership, and will represent those qualified to write on the subject assigned. Each editorial will represent the opinion of the author, and the association will, in no way, be responsible for these expressions of opinion.

COMMITTEE ON FOODS.

Within the past few years our knowledge of the vitamin content, mineral elements and other constituents of foods has increased tremendously. Coincident with this advance there has developed a more thorough appreciation of the use of properly selected foods in the promotion of health. As an editorial writer¹ in the *Journal of the American Medical Association* says: "Diet is recognized as of great importance in the control of diseases affecting the digestive tract and for the management of the degenerative diseases: diseases of the kidneys and of the circulation".

It was but natural for advertising writers to sense the public interest in the matter of food in relation to health and to single out this or that constituent of the raw material for its nutritional value, at times without any knowledge of the changes which it may have undergone in the process of manufacture or preparation.

The American Medical Association, always in the front rank of the protectors of our public health, has just taken a far-reaching and immensely important step in furthering this protection by the creation of a Committee on Foods².

Foods for which health claims are made are to be considered by this committee under rules which are modelled after those effective for "New and Non-official Remedies", and they will not be accepted unless their composition is made known. If claims of special virtue are made for any ingredient, the quantity of that ingredient must be made known, if the committee so requires. False statements regarding ingredients, or methods of preparation, or false and misleading statements of nutritional or health value will be sufficient to bar acceptance. Reports on products approved by the committee will be published in the *Journal of the American Medical Association*, and, at the end of the year, will be published in book form under the title "Accepted Foods".

After acceptance by the committee, the manufacturers of any product may, so long as the product is continued in good standing, use, both on his label and in advertising, the insignia adopted by the Committee on Foods in identifying an accepted product.

COLORIMETRIC METHOD FOR PHOSPHORUS AND ARSENIC.

Chemists that have had experience with colorimetric methods in general, and with the method usually employed for the determination of phosphorus in the presence of silica in particular, have reason to know what a decided advantage the method of

¹ *J. Am. Med. Assoc.*, 93, 1147 (1929).

² *Ibid.*, 1144.

Denigès¹ offers. Attention is directed to the improvement of this method so thoroughly worked out by Truog and Meyer²; as modified it not only takes care of other interfering elements, but it places the method in a position of particular service with respect to the elimination of the effect of silica. From the agricultural chemist's standpoint this is particularly desirable.

The lack of permanency of the color produced by the action of the reducing agent upon the molybdate of phosphorus has proved a hindrance to the complete success of the method, and although the authors have also advanced the technic in this respect, they report but briefly upon attempts to improve the method along this line; furthermore, Parker and Fudge³ find the reaction, under their conditions, permanent for a period of one hour, provided the solution is kept out of the direct rays of sunlight. Further study of this point should prove profitable.

¹ *Compt. rend.*, 171, 802 (1920); *Compt. rend. Soc. biol.*, 84, 875 (1921).

² *Ind. Eng. Chem. (Analytical Ed.)*, 1, 136 (1929).

³ *Soil Sci.*, 24, 109 (1929).

PROCEEDINGS OF THE FORTY-FIFTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1929.

The forty-fifth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 28-30, 1929.

The meeting was called to order by the president, H. B. McDonnell, College Park, Md., on the morning of October 28th, at 10.30 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER, 1930.

Honorary President.

HARVEY W. WILEY, 2345 Ashmead Place, Washington, D. C.

President.

E. M. BAILEY, Agricultural Experiment Station, New Haven, Conn.

Vice-President.

H. D. HASKINS, Agricultural Experiment Station, Amherst, Mass.

Secretary-Treasurer.

W. W. SKINNER, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee.

F. C. BLANCK, Washington, D. C.

J. W. KELLOGG, Harrisburg, Pa.

A. E. PAUL, Chicago, Ill.

H. B. McDONNELL, College Park, Md.

PERMANENT COMMITTEES.

Recommendations of Referees.

(Figures in parentheses refer to year in which appointment expires.)

E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.), *Chairman*.

SUBCOMMITTEE A: A. G. McCall (1930), (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman*; R. N. Brackett (1932); H. H. Hanson (1934). [Waters, brine, and salt; tanning materials and leathers; insecticides and fungicides (fluorine compounds); caustic poisons; soils and liming materials (reaction value of soils, liming materials, less common metals in soils); feeding stuffs (stock feed adulteration, mineral mixed feeds, determination of moisture); sugars and sugar products (honey, maple products, starch conversion products; drying, densimetric, and refractometric methods; polariscopic methods; chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, nitrogen activity methods, high analysis fertilizers, potash); plants (preparation of plant material for analysis, less common metals, total chlorine, carbohydrates, forms of nitrogen); paints, paint materials, and varnishes].

SUBCOMMITTEE B: L. E. Warren (1930), (Food, Drug and Insecticide Administration, Washington, D. C.), *Chairman*; H. C. Lythgoe (1932); A. G. Murray (1934). [Specific gravity and alcohol, naval stores (turpentine); drugs (crude drugs, radioactivity in drugs and water, laxatives and bitter tonics, mercurials, micro-chemical methods for alkaloids, terpin hydrate, santonin, ether, bioassay of drugs, ephedra, thymol, menthol, bromides-chlorides, oil of chenopodium, both salicylates and other phenols in mixtures, small quantities of iodides in mixtures, bismuth compounds in tablets, phenolsulfonates, sulfonal and trional, emetine, chloroform and carbon tetrachloride, guaiacol, calcium lactate, and iodoform; beers, wines, and distilled liquors; colorimetric methods for vitamins].

SUBCOMMITTEE C: H. A. Lepper (1930), (Food, Drug and Insecticide Administration, Washington, D. C.), *Chairman*; J. O. Clarke (1932); C. D. Howard (1934). [Dairy products (milk, butter, cheese, malted milk, dried milk, ice cream, milk proteins, qualitative tests); fats and oils; baking powders and baking chemicals; eggs and egg products (fat lipoids, lipoid P_2O_5 , and total P_2O_5 , detection of decomposition, water-soluble protein, unsaponifiable matter, ash and total solids); food preservatives, coloring matter in foods, metals in foods (arsenic, boron, tin, copper and zinc, and lead), fruits and fruit products (solids in solution of sucrose and organic acids, ash in fruit products, fruit acids), canned foods, vinegars, flavors and non-alcoholic beverages, meats and meat products (separation of meat proteins), gelatin, cacao products (crude fiber, cacao butter), coffee, spices and other condiments, cereal foods (sampling of flour, ash in flour and gasoline color value, glutenin in flour, hydrogen-ion concentration, diastatic value of flour, starch in flour, flour-bleaching chemicals, foreign methods for testing flour, methods for bread analysis—(a) sampling and determination of moisture, (b) lipoids and fat in baked products, (c) milk solids in milk bread, (d) organic and ammoniacal nitrogen in air-dried baked cereal products, (e) crude fiber in alimentary pastes and in air-dried baked cereal products, (f) rye flour in rye bread—experimental baking tests, moisture in alimentary pastes, unsaponifiable matter in flour and in alimentary pastes, water-soluble protein in flour and in alimentary pastes, and collecting and preparing sample of alimentary pastes for analysis)].

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WATERS, BRINE, AND SALT:

General referee: C. H. Badger, Food, Drug and Insecticide Adm., Washington, D. C.

TANNING MATERIALS AND LEATHERS:

General referee: I. D. Clarke, Bureau of Chemistry and Soils, Washington, D. C.

INSECTICIDES AND FUNGICIDES:

General referee: J. J. T. Graham, Food, Drug and Insecticide Adm., Washington, D. C.

FLUORINE COMPOUNDS:

Associate referee: G. A. Shuey, Agricultural Experiment Station, Knoxville, Tenn.

CAUSTIC POISONS:

General referee: J. J. T. Graham.

SOILS AND LIMING MATERIALS:

General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

REACTION VALUE OF SOILS:

a. ALKALINE SOILS:

Associate referee:

b. ACID SOILS:

Associate referee: E. T. Wherry, Bureau of Chemistry and Soils, Washington, D. C.

LIMING MATERIALS:

Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

FEEDING STUFFS:

General referee: V. E. Munsey, Food, Drug and Insecticide Adm., Washington, D. C.

STOCK FEED ADULTERATION:

Associate referee: H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

MINERAL MIXED FEEDS:

Associate referee: H. A. Halvorson, Old Capitol Building, St. Paul, Minn.

MOISTURE:

Associate referee: G. E. Grattan, Department of Agriculture, Ottawa, Can.

SUGARS AND SUGAR PRODUCTS:

General referee: R. T. Balch, Bureau of Chemistry and Soils, Washington, D. C.

HONEY:

Associate referee: H. A. Schuette, University of Wisconsin, Madison, Wis.

MAPLE PRODUCTS:

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STARCH CONVERSION PRODUCTS:

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Associate referee: C. F. Snyder, Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS:

Associate referee: F. W. Zerban, N. Y. Sugar Trade Laboratory, New York City.

CHEMICAL METHODS FOR REDUCING SUGARS:

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FERTILIZERS:

General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID:

Associate referee: W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

NITROGEN:

Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

NITROGEN ACTIVITY METHODS IN FERTILIZERS:

Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

HIGH ANALYSIS FERTILIZERS:

Associate referee: J. B. Smith.

POTASH:

Associate referee: L. D. Haigh, Agricultural Experiment Station, Columbia, Mo.

PLANTS:

General referee: O. B. Winter, Agricultural Experiment Station, E. Lansing, Mich.

PREPARATION OF PLANT MATERIAL FOR ANALYSIS:

Associate referee: H. R. Kraybill, Agricultural Experiment Station, Purdue, Ind.

LESS COMMON METALS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

TOTAL CHLORINE:

Associate referee: M. F. Mason, Agricultural Experiment Station, E. Lansing, Mich.

CARBOHYDRATES:

Associate referee: J. T. Sullivan, Agricultural Experiment Station, Purdue, Ind.

VARIOUS FORMS OF NITROGEN:

Associate referee: H. B. Vickery, Agricultural Experiment Station, New Haven, Conn.

PAINTS, PAINT MATERIALS AND VARNISHES:

General referee: C. S. Ladd, Office of Food Commissioner and Chemist, Bismarck, N. D.

SPECIFIC GRAVITY AND ALCOHOL:

General referee: A. W. Hanson, Food, Drug and Insecticide Adm., Minneapolis, Minn.

NAVAL STORES:

General referee: F. P. Veitch, Bureau of Chemistry and Soils, Washington, D. C.

TURPENTINE:

Associate referee: V. E. Grotlich, Food, Drug and Insecticide Adm., Washington, D. C.

DRUGS:

General referee: A. E. Paul, 1625 Transportation Bldg., Chicago, Ill.

CRUDE DRUGS:

Associate referee: H. W. Youngken, Massachusetts College of Pharmacy, Boston, Mass.

RADIOACTIVITY IN DRUGS AND WATER:

Associate referee: J. W. Sale, Food, Drug and Insecticide Adm., Washington, D. C.

LAXATIVES AND BITTER TONICS:

Associate referee: E. O. Eaton, Food, Drug and Insecticide Adm., San Francisco, Calif.

MERCURIALS:

Associate referee: R. S. Roe, Food, Drug and Insecticide Adm., Chicago, Ill.

MICROCHEMICAL METHODS FOR ALKALOIDS:

Associate referee: C. K. Glycart, Food, Drug and Insecticide Adm., Chicago, Ill.

TERPIN HYDRATE:

Associate referee: C. B. Stone, Food, Drug and Insecticide Adm., Minneapolis, Minn.

SANTONIN:

Associate referee: H. M. Burlage, Oregon Board of Pharmacy, Corvallis, Ore.

ETHER:

Associate referee: H. R. Watkins, Food, Drug and Insecticide Adm., Washington, D. C.

BIOASSAY OF DRUGS:

Associate referee: W. T. McClosky, Food, Drug and Insecticide Adm., Washington, D. C.

EPHEDRA:

Associate referee: C. K. Glycart.

THYMOL:

Associate referee: F. L. Hart, Food, Drug and Insecticide Adm., St. Louis, Mo.

MENTHOL:

Associate referee: F. L. Elliott, Food, Drug and Insecticide Adm., Baltimore, Md.

BROMIDES-CHLORIDES:

Associate referee: N. E. Freeman, Food, Drug and Insecticide Adm., Chicago, Ill.

OIL OF CHENOPODIUM:

Associate referee: L. B. Broughton, University of Maryland, College Park, Md.

BOTH SALICYLATES AND OTHER PHENOLS IN MIXTURES:

Associate referee: F. C. Synkovich, Food, Drug and Insecticide Adm., Chicago, Ill.

SMALL QUANTITIES OF IODIDES IN MIXTURES:

Associate referee: H. B. Mead, Food, Drug and Insecticide Adm., New York, N. Y.

BISMUTH COMPOUNDS IN TABLETS:

Associate referee: J. Calloway, Jr., Food, Drug and Insecticide Adm., New York, N. Y.

COLORIMETRIC METHODS FOR VITAMINS:

Associate referee: H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

PHENOLSULFONATES:

Associate referee: E. H. Grant, Food, Drug and Insecticide Adm., Baltimore, Md.

SULFONAL AND TRIONAL:

Associate referee: W. S. Hubbard, Food, Drug and Insecticide Adm., Baltimore, Md.

EMETINE:

Associate referee: F. C. Synkovich.

CHLOROFORM AND CARBON TETRACHLORIDE:

Associate referee: J. R. Matchett, Prohibition Chemical Labr., 428 Transportation Bldg., Chicago, Ill.

GUAIACOL:

Associate referee: N. T. Knight, Room 204, Old Custom House, St. Louis, Mo.

CALCIUM LACTATE:

Associate referee: E. C. Deal, Food, Drug and Insecticide Adm., New Orleans, La.

IODOFORM:

Associate referee: W. F. Kunke, Food, Drug and Insecticide Adm., Chicago, Ill.

DAIRY PRODUCTS:

General referee: H. C. Lythgoe, Department of Public Health, Boston, Mass.

MILK:

Associate referee: H. Hoffmann, Jr., Dairy and Food Department, Minneapolis, Minn.

BUTTER:

Associate referee: C. W. Harrison, Food, Drug and Insecticide Adm., Minneapolis, Minn.

CHEESE:

Associate referee: E. O. Huebner, Dairy and Food Commission, Madison, Wis.

MALTED MILK:

Associate referee: F. Hillig, Food, Drug and Insecticide Adm., Washington, D. C.

DRIED MILK:

Associate referee: E. L. P. Treuthardt, Food, Drug and Insecticide Adm., Boston, Mass.

ICE CREAM:

Associate referee: R. O. Baird, Food and Drug Laboratory, Bismarck, N. D.

MILK PROTEINS:

Associate referee: H. C. Waterman, Office of Experiment Stations, Washington, D. C.

QUALITATIVE TESTS:

Associate referee: K. E. Wright, Dept. Animal and Dairy Husbandry, Amherst, Mass.

FATS AND OILS:

General referee: G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

BAKING POWDERS AND BAKING CHEMICALS:

General referee: Mayne R. Coe, Bureau of Chemistry and Soils, Washington, D. C.

EGGS AND EGG PRODUCTS:

General referee: S. Alfend, Food, Drug and Insecticide Adm., St. Louis, Mo.

FAT, LIPOIDS, LIPOID P_2O_5 AND TOTAL P_2O_5 :

Associate referee: J. H. Bornmann, Food, Drug and Insecticide Adm., Chicago, Ill.

DETECTION OF DECOMPOSITION:

Associate referee: H. D. Grigsby, Food, Drug and Insecticide Adm., New York, N. Y.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, ASH AND TOTAL SOLIDS:

Associate referee: S. Alfend.

FOOD PRESERVATIVES:

General referee: W. W. Randall, State Department of Health, Baltimore, Md.

COLORING MATTERS IN FOODS:

General referee: C. F. Jablonski, Food, Drug and Insecticide Adm., New York, N. Y.

METALS IN FOODS:

General referee: G. C. Spencer, Bureau of Chemistry and Soils, Washington, D. C.

ARSENIC:

Associate referee: W. C. Taber, Food, Drug and Insecticide Adm., San Francisco, Calif.

BORON:

Associate referee: O. F. Krumboltz, Bureau of Chemistry and Soils, Washington, D. C.

TIN:

Associate referee: A. E. Mix, Food, Drug and Insecticide Adm., Washington, D. C.

COPPER AND ZINC:

Associate referee: R. Walker, Bureau of Chemistry and Soils, Washington, D. C.

LEAD:

Associate referee: E. H. Berry, Food, Drug and Insecticide Adm., Chicago, Ill.

FRUITS AND FRUIT PRODUCTS:

General referee: H. J. Wichmann, Food, Drug and Insecticide Adm., San Francisco, Calif.

SOLIDS IN SOLUTION OF SUCROSE AND ORGANIC ACIDS:

Associate referee: V. B. Bonney, Food, Drug and Insecticide Adm., Washington, D. C.

ASH IN FRUIT PRODUCTS:

Associate referee: G. T. Daughters, Food, Drug and Insecticide Adm., San Francisco, Calif.

FRUIT ACIDS:

Associate referee: B. G. Hartmann, Food, Drug and Insecticide Adm., Washington, D. C.

CANNED FOODS:

General referee: F. C. Blanck, Bureau of Chemistry and Soils, Washington, D. C.

VINEGARS:

General referee: A. M. Henry, Food, Drug and Insecticide Adm., Philadelphia, Pa.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

General referee: J. B. Wilson, Food, Drug and Insecticide Adm., Washington, D. C.

MEATS AND MEAT PRODUCTS:

General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

SEPARATION OF MEAT PROTEINS:

Associate referee: W. S. Ritchie, University of Missouri, Columbia, Mo.

GELATIN:

General referee: R. M. Mehurin, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:

General referee: J. W. Sale, Food, Drug and Insecticide Adm., Washington, D. C.

CRUDE FIBER:

Associate referee: Marie L. Offutt, Food, Drug and Insecticide Adm., New York, N. Y.

CACAO BUTTER:

Associate referee: M. M. Jackson, Food, Drug and Insecticide Adm., New York, N. Y.

COFFEE:

General referee: P. A. Clifford, Food, Drug and Insecticide Adm., Washington, D. C.

SPICES AND OTHER CONDIMENTS:

General referee: K. C. Beeson, State Chemical Laboratory, Vermilion, S. D.

CEREAL FOODS:

General referee: J. A. LeClerc, Bureau of Chemistry and Soils, Washington, D. C.

SAMPLING OF FLOUR:

Associate referee: H. Runkel, Food, Drug and Insecticide Adm., Chicago, Ill.

ASH IN FLOUR AND GASOLINE COLOR VALUE:

Associate referee: D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C.

GLUTENIN IN FLOUR:

Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

HYDROGEN-ION CONCENTRATION OF FLOUR:

Associate referee: C. H. Bailey, University of Minnesota, Minneapolis, Minn.

DIASTATIC VALUE OF FLOUR:

Associate referee:

STARCH IN FLOUR:

Associate referee: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C.

FLOUR-BLEACHING CHEMICALS:

Associate referee: G. C. Spencer, Bureau of Chemistry and Soils, Washington, D. C.

FOREIGN METHODS FOR TESTING FLOUR:

Associate referee: C. H. Bailey.

METHODS FOR BREAD ANALYSIS:

a. SAMPLING AND DETERMINATION OF MOISTURE:

Associate referee: L. H. Bailey.

b. LIPOIDS AND FAT IN BAKED PRODUCTS:

Associate referee: J. H. Bornmann, Food, Drug and Insecticide Adm., Chicago, Ill.

c. MILK SOLIDS IN MILK BREADS:

Associate referee: L. H. Bailey.

d. ORGANIC AND AMMONIACAL NITROGEN IN AIR-DRIED BAKED-CEREAL PRODUCTS:

Associate referee: S. C. Rowe, Food, Drug and Insecticide Adm., Washington, D. C.

e. CRUDE FIBER IN ALIMENTARY PASTES AND IN AIR-DRIED BAKED-CEREAL PRODUCTS:

Associate referee: R. L. Horst, Food, Drug and Insecticide Adm., New Orleans, La.

f. RYE FLOUR IN RYE BREAD:

Associate referee: C. B. Stone, Food, Drug and Insecticide Adm., Minneapolis, Minn.

EXPERIMENTAL BAKING TESTS:

Associate referee: M. J. Blish.

COLLECTING AND PREPARING SAMPLE OF ALIMENTARY PASTE FOR ANALYSIS:

Associate referee: S. C. Rowe.

MOISTURE IN ALIMENTARY PASTES:

Associate referee: S. C. Rowe.

UNSATURIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES AND WATER-SOLUBLE PROTEIN IN ALIMENTARY PASTES:

Associate referee: S. Alfend.

BEERS, WINES AND DISTILLED LIQUORS:

General referee. W. V. Linder, Bureau of Internal Revenue, Washington, D. C.

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Adams, J. R., Bureau of Chemistry and Soils, Washington, D. C.

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 Frary, G. G., State Department of Agriculture, Vermilion, S. D.

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Gifford, R. S., American Cyanamid Co., Linden, N. J.
Gowen, P. L., Campbell Soup Co., Camden, N. J.
Graham, J. J. T., Food, Drug and Insecticide Administration, Washington, D. C.
Grant, E. H., Food, Drug and Insecticide Administration, Washington, D. C.
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Grewe, E., Bureau of Dairy Industry, Washington, D. C.
Griem, W. B., Department of Agriculture and Markets, Madison, Wis.
Griffin, E. L., Food, Drug and Insecticide Administration, Washington, D. C.
Grigsby, H. D., Food, Drug and Insecticide Administration, New York City.
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PRESIDENT'S ADDRESS¹

EXPERIMENTS WITH OZONE ON GUINEA PIGS AT THE JAMES TODD LABORATORY OF THE UNIVERSITY OF MARYLAND.

By H. B. McDONNELL (University of Maryland, College Park, Md.).

Great interest has been shown by chemists recently in regard to elements and compounds which occur in small quantities, but which have been found to be, or may be, of great importance to plants or animals, or both. I venture to call your attention to ozone, one of these elements that has not received consideration from this association. For several years I have been working with ozone, mostly in low concentrations, and noting its results on guinea pigs. Therefore I shall explain briefly some of the experiments performed.

This modification of the element oxygen has been known for about a century and has been the subject of extensive investigation and more speculation. Many popular articles have been written in regard to the wonderful curative and health-giving properties of the ozone in the air, but few data have been given to substantiate the claims made.

A notable research to determine the total quantity of ozone in the atmosphere has been in progress for several years under the auspices of the Royal Society of London². Special spectrographs were designed to determine the quantity of ozone over any given place, and many observations were made in different parts of Europe. While the amounts found varied considerably at various times and places, the average was equal to a layer of pure ozone nearly 3 mm. thick at normal temperature and pressure, or equal in weight to nearly 5 mm. of air at ordinary temperature and pressure. If this quantity were distributed evenly in the first mile of the atmosphere it would give at least 40 parts per million of ozone, by weight, enough to destroy all the higher animals in a few days, if not a few hours. Fortunately most of this ozone is many miles above the earth's surface, where the air is very cold and dry and where the ultra-violet rays of the sun are abundant. This ozone layer serves as a screen to protect the bulk of the atmosphere and the earth's surface from the withering effect of too much ultra-violet light.

However, from the standpoint of practical agriculture, we are more interested in the quantity of ozone at or near the surface of the earth and its effect on animals and plants.

¹ Presented Tuesday morning, October 29th, as special order of business for 11 o'clock.

² Proc. A 110, A 114, and A 122.

The recent popularity and extensive use of ultra-violet light adds a new angle and new importance to ozone. This light generates ozone. To what extent are the effects due to ozone? In a number of cases this question has been answered. Thus, in the irradiation of ergosterol by ultra-violet light, the vitamin content is increased rapidly at first, but it soon diminishes. We are told that the vitamin is produced by the direct actinic rays and not by the ozone. But what causes the destruction of the vitamin?

I exposed a 1 per cent solution of potassium iodide in a photographic tray to the action of a mercury arc lamp placed 10 inches away for 10 minutes. Iodine was set free.

The experiment was repeated a number of times in the same way, and it was found that the amount of iodine liberated varied widely. When the revolutions of the disc of the wattmeter in a given time were counted it was found that owing to the irregularities of the arc or of the rectifier, or both, the number varied as much as 50 per cent within a short time. When the solution was placed in a transparent silica tube about 3 cm. in diameter and 25 cm. long, closed at one end and drawn down to a 3 mm. opening at the other, and exposed to ultra-violet light there was a very slight liberation of iodine, and when the solution was boiled and placed in the tube while hot, and the end was closed with a short piece of rubber tubing with clamp, allowed to cool and exposed as before, no iodine was liberated. Thus, in the case of potassium iodide, it is the ozone and not the light that causes the chemical reaction. A somewhat similar experiment, but one in which a sparking generator, which gives more oxide of nitrogen, was used, was reported by Ross¹.

An example of the production of a compound by ozone and its destruction by an excess of ozone, is its action on the loco, or reduction compound, of flourescene. When this compound was treated with ozone, flourescene was again formed, but it was destroyed by an excess, a colorless compound resulting. Both of these reactions, which are quantitative, were used by Egorow in determining ozone colorimetrically².

The beneficial effects of ultra-violet light are believed by some scientists to be caused by its action on the skin to form compounds of the nature of vitamins. Is it not possible that ozone may so act on the skin as to produce similar results if the concentration and time of application are correct?

The quantity of ozone in the air near the surface of the earth evidently varies, but certainly this variation is not so great as some of the published figures would indicate, because it is very difficult to determine

¹ Ellis and Wells. *Chemical Action of Ultra-violet Rays*, p. 129.

² *Z. Untersuch. Lebensmittel.*, 4, 56, 858 (1928).

such small amounts with accuracy. It has been stated recently by Dadlez¹ that the amount of ozone in the air is generally between 0.01 and 0.02 mg. per cubic meter. I have tried repeatedly to get a test for ozone in air by drawing 1 cubic meter of air through a solution of potassium iodide, but I could not get a trace of blue color with starch. However, the smallest amount of ozone that can be determined by this method, with a probable error of ± 0.01 mg., is 0.1 mg. No acid should be added to the potassium iodide solution, as even slight acidity will cause liberation of iodine in a short time even when no air is passed through the solution.

Ordinary gas meters equipped with special unit-reading dials are inexpensive, and they serve very well to measure the volume of air in such tests. I have used them extensively for testing artificially ozonized air, which is drawn through by some form of aspirator.

Owing to the destructive action of ozone on rubber, this material cannot be used for connecting glass tubes except in the case of lower concentrations of ozone, for example 1 or 2 parts per million, and then the rubber should be lubricated with glycerol and the connection made as close as possible. For higher concentrations the brass unions used so extensively for connecting small copper pipe on automobiles, etc., may be employed, but with glass tubing the circular brass packing wedge should be removed and asbestos thread used instead.

Any ozonizer in which air is used will be much more effective if the moisture is removed from the air before it is ozonized. A U. S. Ozone Company's generator, which gave 5 mg. of ozone per liter when a silica gel drier was used, yielded only half this quantity when the drier was not used.

When air is ozonized, nitrogen peroxide is formed, but it is in such a very small amount that it can generally be disregarded. Tests of this action reported several years ago on two types of commercial ozonizers gave, on the average, an approximate ratio of ozone to nitrogen oxidized of 150 to 1².

Approximately 20 years ago Mr. James Todd of Pittsburgh became interested in the treatment of disease, especially tuberculosis, by the use of ozone in the form of ozonized air. The general idea was not new, as medical and popular publications contained many essays in regard to the curative properties of ozone. However, most of these essays were of a popular rather than a scientific nature. Small generators of ozone to be used in purifying air in ventilation and in treating respiratory troubles were on the market.

¹ *Union pharm.*, 69, 17 (1928); *C. A.*, 23, 2660 (1929).

² *J. Am. Soc. Heating. Ventilating Eng.*, March, 1922, p. 191.

Mr. Todd conducted extensive experiments at his factory near Pittsburgh, using ozonized air on guinea pigs that had been inoculated with tuberculosis, and later published descriptions of these experiments and the results as he interpreted them in a book called "Experiments with Oxygen on Disease", the last edition of over 300 pages being published in 1921. What appears to be very remarkable results were obtained in the early work, as shown by the chart on page 237 of his book. No details are given as to how the animals were kept or the amount of ozone in the test rooms.

After experimenting with ozone and ozonized oils, especially cod liver oil, for a number of years, Mr. Todd, in 1922, interested the authorities of the University of Maryland in his work. With Mr. Todd's financial assistance, they fitted out the Vaccine Building, recently acquired, for extensive experiments to be conducted according to Mr. Todd's plans and with his machinery.

This building was named the James Todd Laboratory. It is a one-story building, with a frontage of 106 feet. The central portion, which is 30 x 28 feet, contains offices and laboratories for the chemist and pathologist. A south wing, 38 x 24 feet, furnishes an animal room, and for some experiments the "check" or "no ozone" animals were kept here. The north wing, 38 x 24 feet, has two 9 x 10 foot rooms, the one at the north end being used to prepare ozonized oils, generally cod liver oil, and the other, Room No. 4, being used in several of the experiments for treating guinea pigs with ozone. Along the west wall of this north wing are rooms No. 1, No. 2, and No. 3. Room No. 1 is 8 x 7½ feet and 9 feet high, and the west wall of the building forms its west wall. Rooms Nos. 2 and 3 are similar to No. 1, but they have no outside main wall. These three rooms are lined with sheet iron and painted, and each has about 500 cubic feet capacity. Room No. 4 has over 1200 cubic feet capacity and has two windows on the west side and one on the north side; while it has a wall radiator, it has a north-west exposure and gets colder than the other rooms in severe winter weather. Rooms Nos. 1, 2 and 3, having no radiators, get their only heat from the main room. The remaining space in the north wing is taken up by the machinery used for generating and distributing ozonized air and also furnishing pure air to these rooms.

Starting with the commercial alternating current through a switchboard with voltmeter and ammeter, there is a 5 horsepower motor driving a shaft to which are belted (1) a Root blower to furnish fresh air to the experimental rooms, (2) a 1 kilowatt alternating current generator, with rheostat on the switchboard in series with the field coils (this arrangement was used instead of the direct current from the com-

mercial lines in order to give a wider range of voltage, the normal voltage being 110), (3) two Crowell pumps for sampling purposes, and (4) a countershaft driven by cone pulleys with a choice of four speeds, to drive either of two Root blowers used to draw or push air through the ozonizer and into the test rooms, the amount being regulated by distributors in the test rooms. The pressure was kept constant by a waste valve that allowed the excess of air or ozonized air to pass into the outside air. The distributors used for the first part of the work, including Experiment D, are shown in Mr. Todd's book on page 309. These distributors have a series of holes that have $1/100$ the capacity of the opening from the fresh air pipe. The number of openings in use is regulated by a sliding sleeve. One hole gave 1 per cent gas or ozonized air, 2, 2 per cent, etc.

The ozone generator is Mr. Todd's design, and it is similar to the one used by him in Pittsburgh. It consists of a box 3 x 3 feet and 27 inches high, made of boiler plate, and has a false bottom with four 9 inch square holes, over each of which is placed a generating cell for ozone. These cells are made of heavy slate, 9 by 9 inches in the clear, and 13 inches high, open at top and bottom. They were filled with corrugated electrodes, generally of aluminum, 7 x 10 inches, with fiber binding on the sides $\frac{1}{8}$ inch thick. Each cell contained 28 of these electrodes, alternating with panes of glass about $\frac{1}{8}$ inch thick. Alternate electrodes were connected with the two high tension wires from the transformer. The usual voltage was about 12,000, but since the cells were connected two and two in parallel series, the voltage on each cell was about 6,000. This voltage, however, was varied in some of the experiments.

The generator box had 6 inch openings in the top and bottom, but these were at once reduced for 4 inch iron pipe; one was connected with the blower, the other with the experimental rooms and outside air. For the electrode experiments a separate 1 inch iron pipe was run from the top of each of the four generating cells to one of the experimental rooms corresponding in number.

The concentration of ozone desired at the generator was from 80 to 100 parts per million. However this was generally reduced considerably as it passed through the iron pipes to the various rooms. The usual volume of air through the generator was about 100 cubic feet per minute.

The U. S. Ozone Company furnished one of their generators, equipped with silica gel driers, which was used in several of the later experiments and also in preparing some of the ozonized oil used in outside work. This machine, when set to deliver 1-cubic foot of air per minute and with

driers in good condition, gave a concentration of ozone of about 5 mg. per liter.

PERSONNEL.

Mr. Todd was at the head of the work. He designed the machinery and plant, and except during a short period outlined the experiments and formulated the rules and methods. Dr E. M. Pickens, head of the Department of Bacteriology and Sanitation, was in charge. I was in immediate charge of plant and machinery operation, of the sampling and testing of the air in the rooms, of pipes, etc., for ozone strength, and of the daily machinery and weather records. Drs. Jacobi, Crawford, VanVlete and DeVolt, in succession, had charge of the weight records, weekly reports, curve sheets and post-mortems.

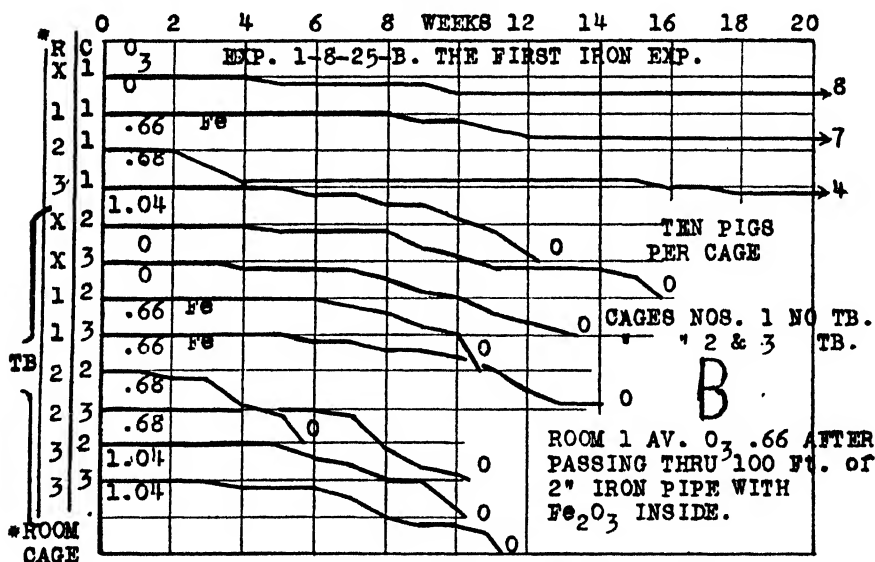


Chart B.—In this experiment there were three cages of 10 guinea pigs each in each of the four rooms. All animals in a room received the same ozone treatment. Room X received no ozone, Room 1 received an average of 0.66 part of ozone per million parts of air, by weight, the ozonized air being passed through a 2-inch iron pipe with additional ferric oxide inside. This was done to see if such treatment rendered the remaining ozone less irritating. Room 2 received practically the same amount of untreated ozone, while Room 3 received about 50 per cent more of the untreated ozone. There were two cages of inoculated pigs in each room, each being inoculated with a different strain of tuberculosis germs.

The first regular comparative experiment was *Experiment 6-12-24-A*. (This nomenclature indicates the month, day and year the experiment was started.) All the guinea pigs were inoculated subcutaneously with human tuberculosis germs. Eighty pigs were kept in three wire pens on the lawn. They had small box shelters in the pens. Rooms Nos. 1, 2 and 3 contained 30 pigs each and received ozonized air averaging nearly 1 part per million, but varying considerably. Analyses for ozone

were made twice daily. Room No. 4, containing 25 pigs at the start, received no ozone. The mortality record is charted, and the weekly weights are available, but they are not included.

In the following experiments all the guinea pigs were kept in the building.

One experiment, *Experiment 1-8-25-B*, was called the "First Iron Experiment", as the ozonized air to Room No. 1 was passed through 100 feet of 2 inch iron pipe with iron oxide placed inside. The hope was that this would remove the irritating gases which caused pneumonia in many of the guinea pigs. The iron treatment removed about half of the ozone delivered to the pipe to Room 1, but as twice as much was delivered as to Room 2 the concentration in the rooms was approximately equal. Room No. 3 received about 50 per cent more ozone than

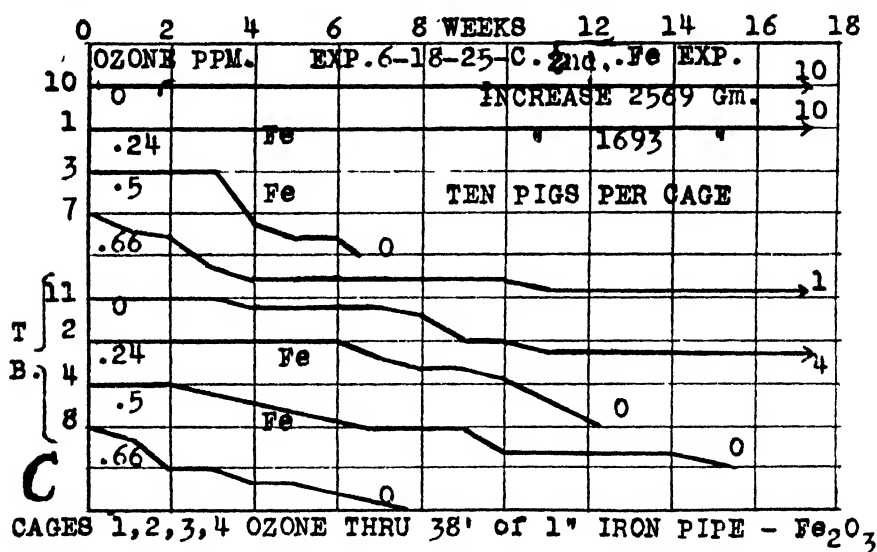


Chart C.—This was called the "Second Iron Experiment", as the ozonized air to two of the rooms was passed over ferric oxide placed in the supply pipes to these rooms, which contained Cages 1 and 2, with an average of 0.24 part per million, and Cages 3 and 4, which received 0.5 part, or practically twice as much. The room with Cages 7 and 8 received an average of 0.66 part of ozone per million, but it was not treated with iron oxide. Cages 5, 6 and 9 were used in this experiment, but the pigs were inoculated at a different time and do not fit into this chart. The cage numbers are placed in the left-hand column. All pigs in Cages 10 (no ozone) and 1 (0.24 p. m. ozone) were alive at the end of the experiment—17 weeks, but the former had gained 2569 grams and the latter only 1693 grams.

Nos. 1 and 2; Room X received no ozone. All the pigs were in cages, 10 animals in each cage. Each room contained three cages: No. 1, not inoculated, Nos. 2 and 3 inoculated with different strains of tuberculosis germs. The mortality results are given in Chart B.

Experiment 6-18-25-C was the "Second Iron Experiment". The ozonized air was passed through 38 feet of 1 inch iron pipe to Rooms 1, 2 and 3. The pipes to Rooms 1 and 2 had $\frac{1}{4}$ pound of iron oxide

placed inside and distributed along about half of the length of the pipe. In this experiment the ozonized air was passed through the iron pipes much more rapidly than in the previous experiment, as the pipes had only $\frac{1}{4}$ the cross section and about $\frac{1}{3}$ the length, or about $\frac{1}{12}$ the capacity. Rooms 1, 2 and 3 received ozone, Room X, no ozone. The pigs in the first cage in each room, Cages 1, 3, 7 and 10, were not inoculated; the second cages, 2, 4, 8 and 11, were inoculated. Pigs in Cage 5, Room 1, were inoculated and kept out of the ozone for two weeks; those in Cage 6, Room 2, were kept in the ozone two weeks before inoculation; those in Cage 9, Room 3, were kept out of ozone for two weeks, then inoculated.

The mortality record is given as Chart C, which does not include the pigs inoculated before or after the beginning of the experiment. It will be noted that the uninoculated pigs in the low-ozone room and in the no-ozone room were all alive at the end of 17 weeks, but that the former had gained 1693 grams and the latter 2596 grams per cage of ten pigs.

Experiment 12-24-25-D was the "First Electrode Experiment". This was to test different forms, materials and areas of electrodes. One inch iron pipes were run from immediately above each generating cell, numbered 1, 2, 3 and 4, to the corresponding rooms. Each iron pipe contained $\frac{1}{4}$ pound iron oxide, distributed.

The electrodes used were as follows:

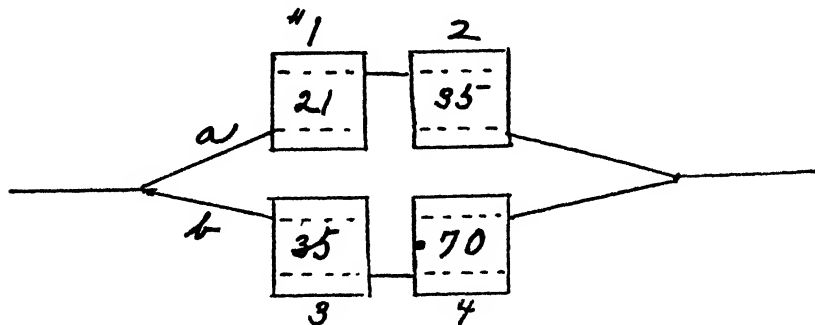
Room 1, Cell 1, Electrodes 3 x 7 in. = 21 sq. in.—corrugated iron.

Room 2, Cell 2, Electrodes 5 x 7 in. = 35 sq. in.—aluminum, mattress type.

Room 3, Cell 3, Electrodes 5 x 7 in. = 35 sq. in.—corrugated aluminum.

Room 4, Cell 4, Electrodes 10 x 7 in. = 70 sq. in.—corrugated aluminum.

Each cell contained 28 electrodes. The cells were connected as usual according to the following diagram:



The squares represent the generating cells, the figures inside, the approximate relative areas of the electrodes used. Circuit a, with an

electrode area about $\frac{1}{2}$ that of circuit b had twice the resistance and got $\frac{1}{2}$ of the current (milliamperes); while Cells 1 and 2 differed in resistance, each received the same amount of current and gave, approximately, the same amount of ozone, but this was about half as much ozone as Cells 3 and 4 on circuit b.

The four 1 inch pipes carried but a small part of the ozonized air, the balance being carried off by the large iron pipe leading from the center of the top of the iron box of the ozonizer.

It is difficult to draw conclusions from this experiment. Pneumonia played havoc with the pigs. This was made worse than usual, I am sure, by the near-zero weather that came at the end of the first week. The steam heat was not kept up during the night and a blast of cold, outside air was blown into all the rooms except Room 5, the "check" room with no ozone. Why this epidemic should have been worse in Cage 4, where 8 pigs died the first week and the other two a few days later, I do not know. I do not think that the slight difference in the construction of the electrodes had anything to do with it. The mortality record is given in Chart D.

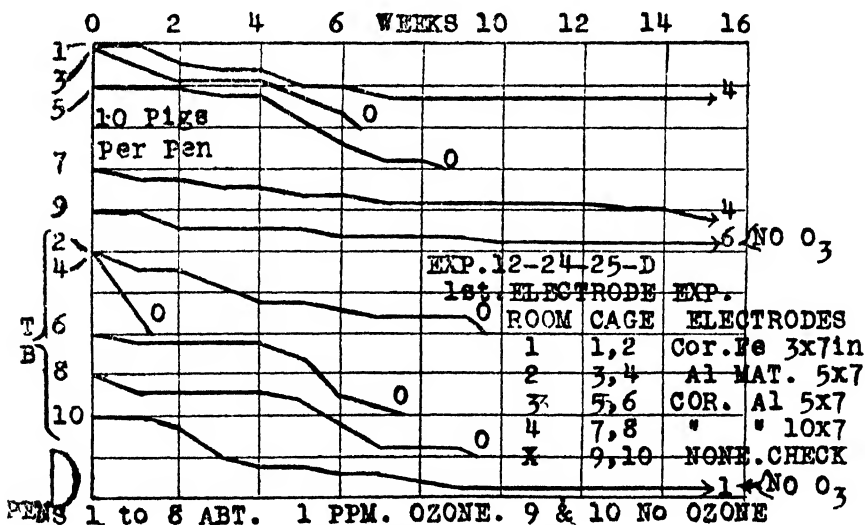


Chart D.—In this experiment there were four ozone rooms with practically an average of 1 part per million of ozone, and a fifth room, X, with no ozone. Each of the four ozone rooms received its supply from a different cell of the generator, since the cells were equipped with electrodes of different size, form or material, as noted on the chart. The experiment was started on December 24, but it was of little value because the severe cold at night caused excessive mortality.

Experiment 4-14-26-E was the "Second Electrode Experiment". This was a repetition of Experiment D, which was not satisfactory, owing, no doubt, to very cold weather. The electrodes and pipes remained as

in the previous experiment. The ozone concentration was to be, as near as possible, 0.5 part per million. New ozonized-air distributors, to give better facilities for regulation, were installed, and the fresh air inlets were reduced to 1 inch, or about $2/7$ of the capacity of the old ones. Only seven pigs were available for each cage. In all the other experiments, except A, 10 pigs per cage was the rule. The results were more regular and satisfactory than in Experiment D.

The uninoculated animals in Cage 3 showed the best results in the ozone-treated cages, all being alive at the end of 17 weeks. However, Cage 9 (no ozone) had the same record, with a greater increase in weight. The increase for Cage 3 was 868 grams, and for Cage 9 it was 2258 grams. However, the inoculated pigs in Cage 3 did not have so good a record as the inoculated ones in the other cages. Taken as a whole, there was no marked difference in the records of all the ozone cages, but the no-ozone cages were slightly better.

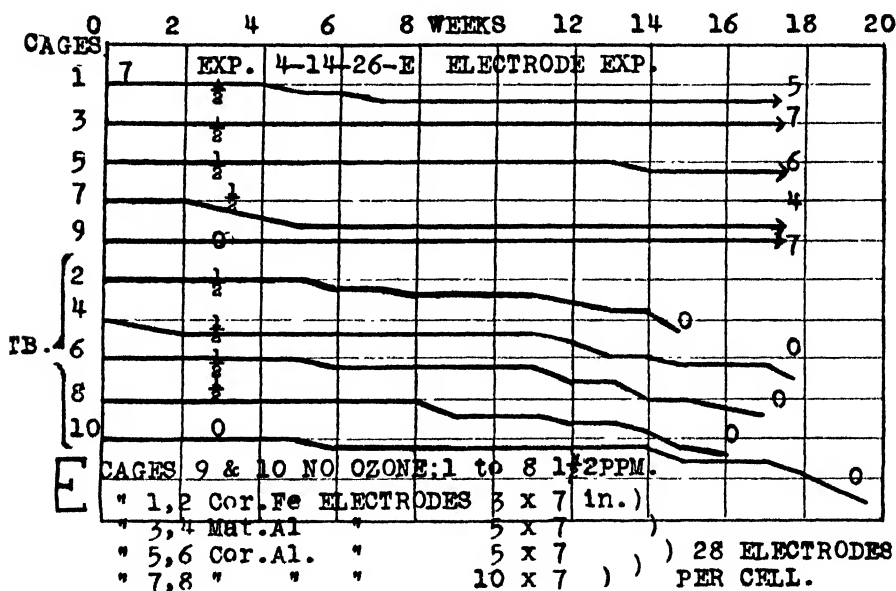


Chart E.—This experiment was a repetition of Experiment D, except that only half as much ozone was used, and 7 pigs were placed in a cage instead of 10. The results seem to indicate that neither the form nor material of the electrode, nor even the voltage used, had much effect on the result. The difference in the resistances of the different cells caused a difference in the voltage used on that cell, as explained above. This experiment ran for 17 weeks.

This experiment does not indicate that the form or material of the electrodes, or the voltage at which the ozone was generated had anything to do with the results.

For the tabulated record, see Chart E.

Experiment 3-31-27-F.—The experiments up to this time indicated that the ozone concentration was too strong and that it shortened the lives of the guinea pigs, both inoculated and uninoculated. The ozone concentration, therefore, was reduced for this experiment to $\frac{1}{4}$ part per million in Room 1 and $\frac{1}{2}$ part per million in Room 2. Room No. 3 received no ozone. Two cages of ten pigs each were placed in each room. The first, or odd-numbered cages, contained pigs not inoculated; the second, or even-numbered cages, contained the animals inoculated with tuberculosis. All the inoculated animals, except one in Cage 6, with no ozone, were dead in 14 weeks. Cage 4, with 0.5 part per million of ozone, showed a slightly better record than No. 2, with half as much ozone, but the difference is too small to have any special significance.

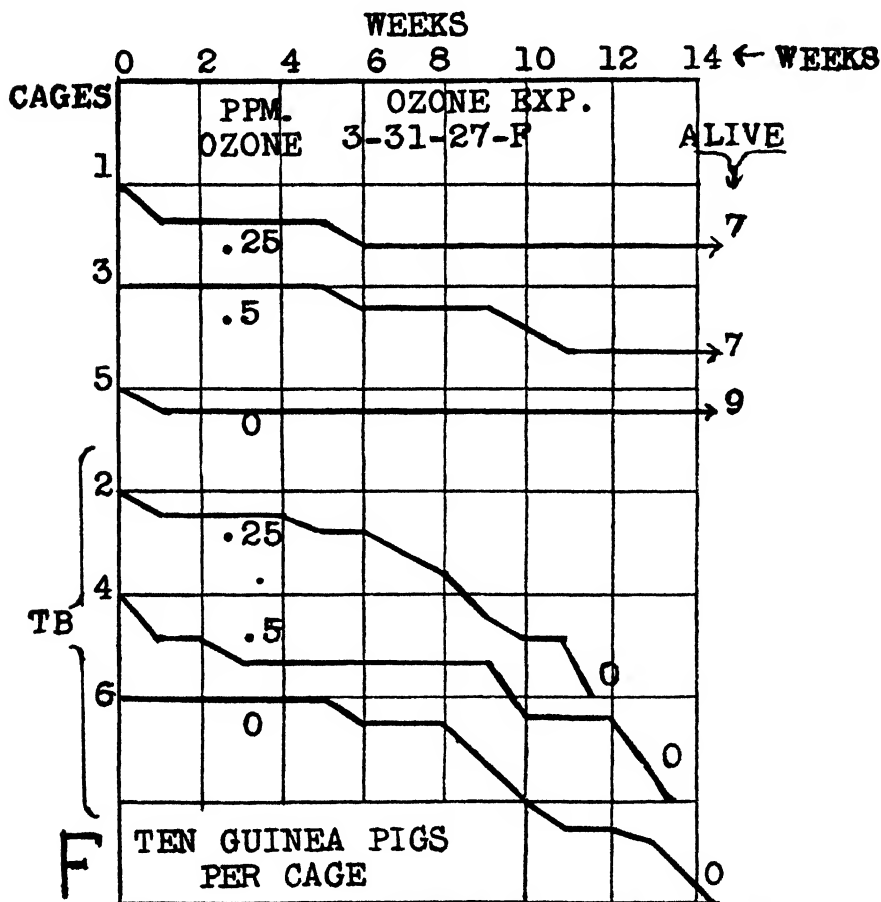


Chart F.—In this experiment but two ozone rooms were used with two cages of 10 pigs each in a room. Cages 1 and 2 received an average of 0.25 p. p. m. of ozone and 3 and 4 received 0.5 part. Nos. 5 and 6 received no ozone. Odd numbers were checks, while even numbers were inoculated. The experiment ran for 14 weeks, as the last inoculated pig died within two days of this time.

It is not quite so good, however, as Cage 6 with no ozone. A similar ratio holds with the uninoculated animals, though the mortality was, of course, much less, 7, 7 and 9 pigs being alive at the end of 14 weeks in Cages 2, 4 and 6, respectively. For the mortality record, see Chart F.

Experiment 11-14-27-G was similar to F, except that the ozone was further reduced to 0.2 part per million in Room 2 and 0.4 part in Room No. 3. Room No. 1 received no ozone. The U. S. Ozone Company's machine was used for the first time in these experiments. It was

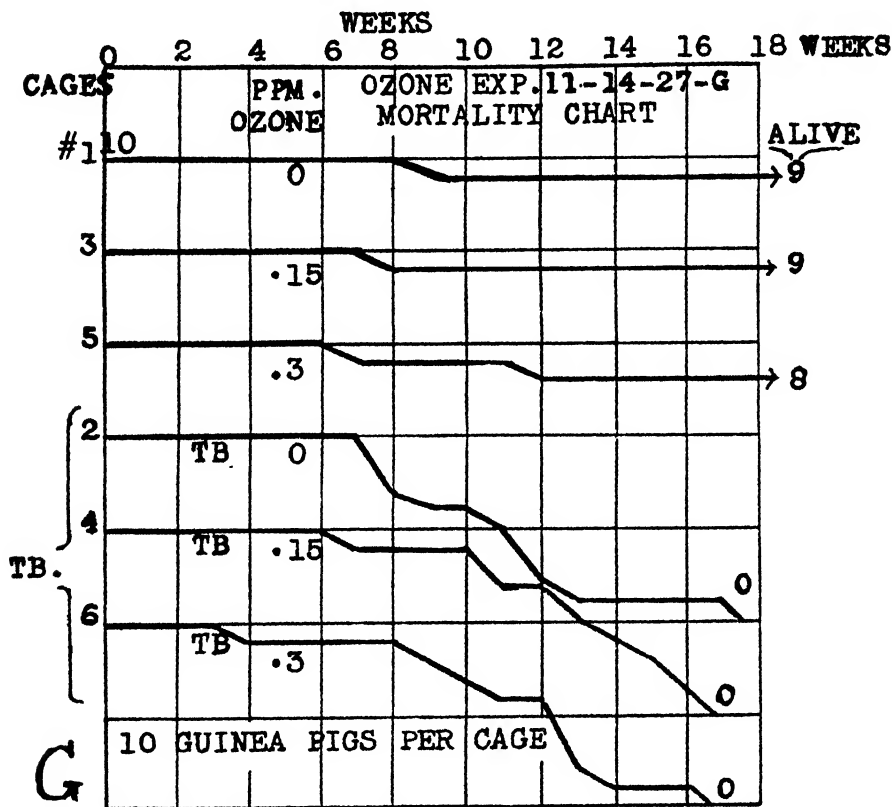


Chart G.—This experiment was similar to the previous one, except that the amount of ozone was further reduced. The pigs in Cages 1 and 2 received no ozone, those in 3 and 4 an average of 0.15 and 5 and 6 an average of 0.3 part per million, respectively. This small amount of ozone had but little effect on the pigs during the experiment—18 weeks.

equipped with duplicate silica gel driers for the air used, and it gave a regular output. The transformer wires were connected to give the smallest output of ozone, or about 1/30 of its maximum capacity. It was seldom necessary to open the relief valve. The pressure on both ozone and air lines was reduced, and the voltage was regulated slightly with the rheostat. The ozone concentration varied only slightly from the desired amount. For the mortality record, see Chart G.

Experiment 4-12-28-H was similar to G, the previous one, except that the ozone concentration was but half as much, namely 0.1 part per million in Room 2, and 0.2 part per million in Room 3. The low concentrations of ozone used in Experiments G and H had little or no effect on the mortality of the pigs during the experiment proper. However, the uninoculated pigs from Experiment H, 9 in each cage, were kept till December 13, 1928, eighteen weeks after removing the cages to the general animal room, and the number alive in Cages 1, 3 and 5 was 9, 7 and 3, respectively. Cage 1 received no ozone, Cage 2, 0.1 and Cage 3, 0.2 part per million ozone. See Chart H.

In addition to the records given in the accompanying charts, weekly

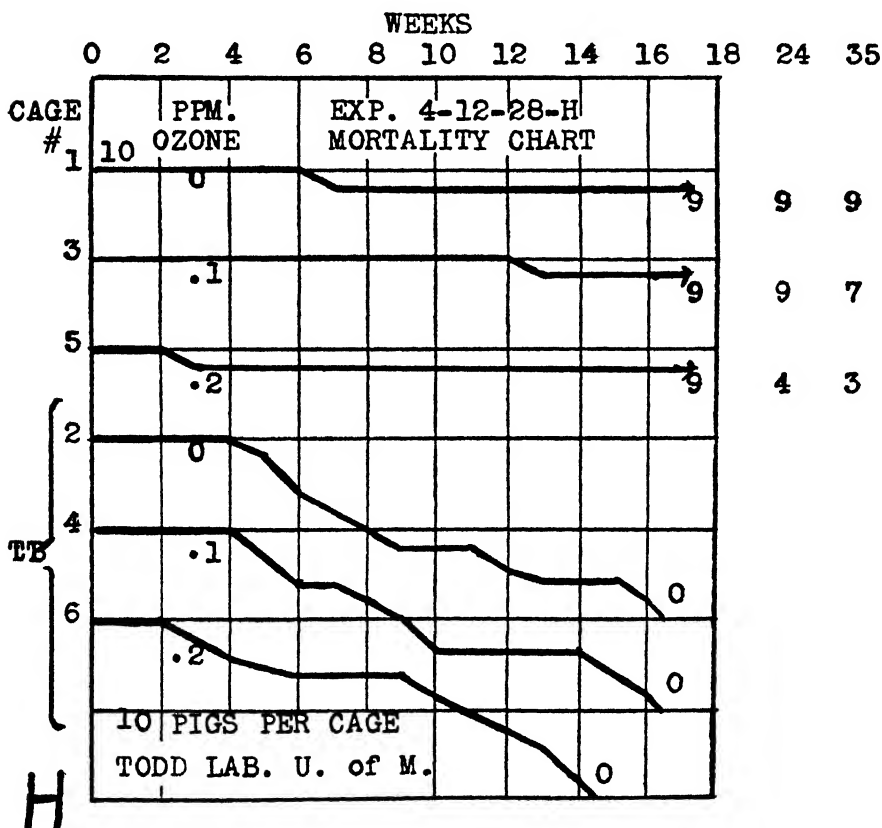


Chart H.—Experiment H was similar to Experiments F and G, except that the concentration of ozone was further reduced to but 0.1 p. p. m. for Cages 3 and 4 and 0.2 part for Cages 5 and 6. This experiment also indicated that for the length of time of the ozone treatment and of the experiment proper, the small amount of ozone had little effect. However, the check, or uninoculated cages, containing 9 surviving pigs each at 17 weeks, were kept without further ozone till the end of the 35th week. At 24 weeks there were 9, 9 and 4 survivors and at 35 weeks 9, 7 and 3 in the cages receiving no ozone, 0.1 part and 0.2 part per million of ozone, respectively. This suggests that even these small concentrations of ozone irritate the lungs of the pigs sufficiently to cause an increased susceptibility to pneumonia.

weight records, post-mortem records, temperature, voltage and relative humidity records were kept.

No one has demonstrated that there are different forms of ozone with different properties. It is extremely poisonous when inhaled in higher concentrations because it acts on the mucous membrane and forms a froth, which, when the ozone is in a concentration as great as one part by weight in 1000, will cause the death of guinea pigs in a short time by filling the lungs and cutting off the air supply. In lower concentrations, down to a few parts per million, pneumonia often develops, causing death sooner or later, depending on the concentration.

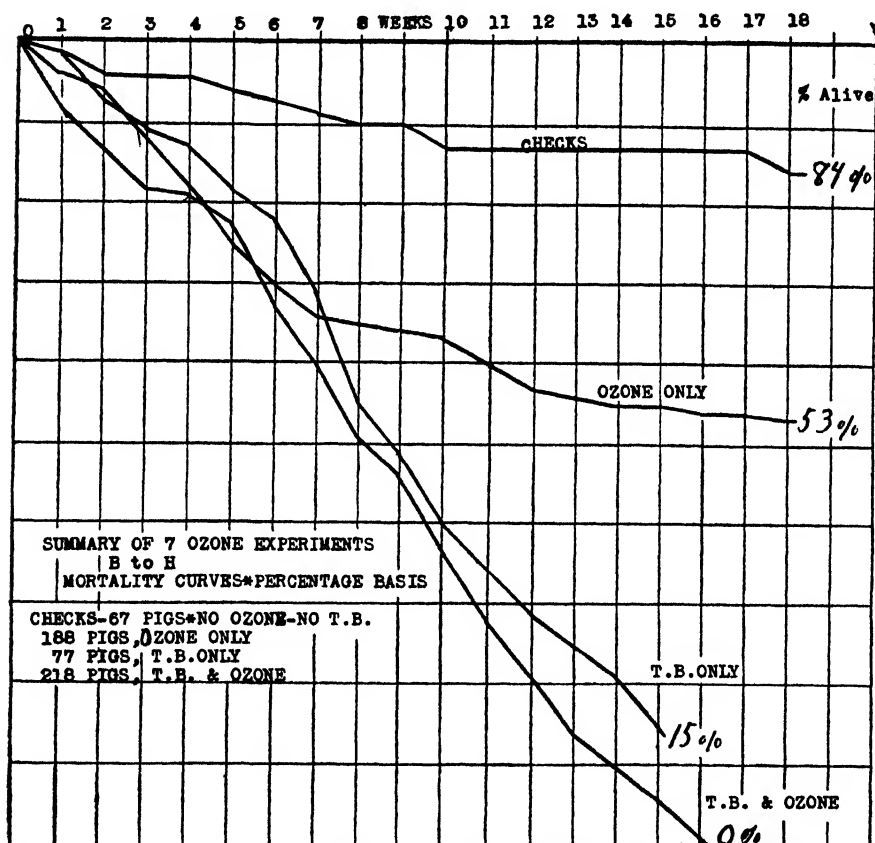
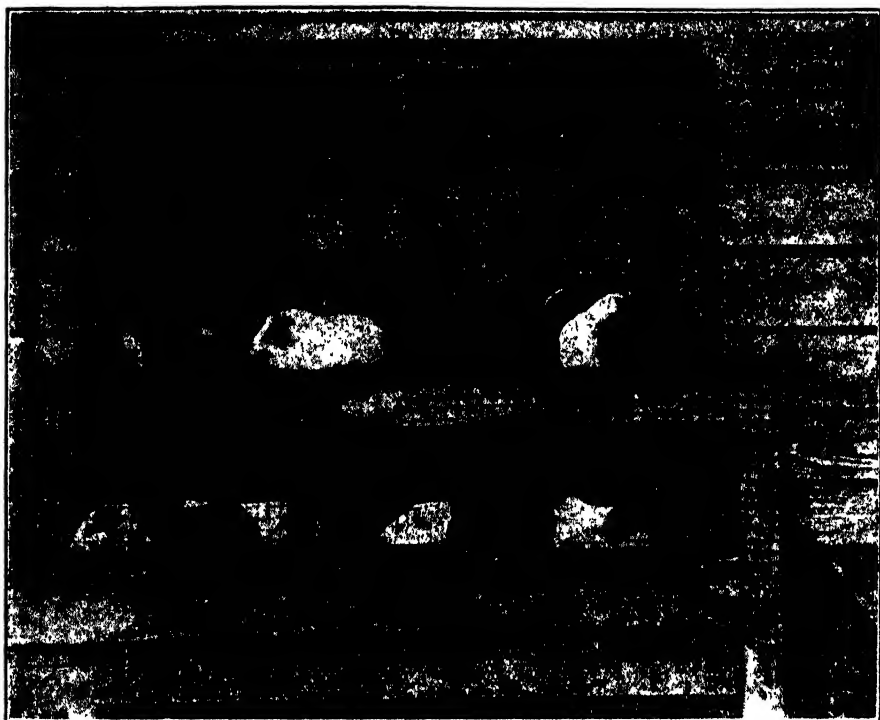


Chart X is a summary of the seven experiments charted and described. It shows the percentage of survivors at the end of each week, up to 18 weeks, for the uninoculated pigs. Each line starts at 100 per cent. The upper line represents the results with 67 pigs not inoculated and with no ozone, 84 per cent being alive at 18 weeks. The next line shows the result from 188 pigs treated with ozone only, with 53 per cent alive at 18 weeks. The next line shows the results from 77 inoculated pigs, with no ozone, 15 per cent being alive at 15 weeks. The last line shows the results from 218 pigs inoculated and treated with ozone, all being dead at 16 weeks. All ozone treatments were continuous night and day during the experiments.



Experiment 4-12-28-H.—Cage 1, Room 1.—October 18, 1928. After 27 weeks, 9 pigs living.

The work seems to demonstrate that ozone given continuously in the respired air for several months, in concentrations even less than one part per million, by weight, apparently shortens the lives of guinea pigs, whether tubercular or disease-free, by irritating the lungs and bronchial tubes and causing pneumonia. The highest concentration that is harmless has not been worked out. To do this the ozonized air should be given for several months, the animals should be under observation for at least a year, and the results should be carefully compared with similar check animals.

Charts, lettered to correspond with the experiments noted, are given. Each heavy line in Charts B to H represents a cage of 10 guinea pigs at the start, except Chart E, which represents but 7 pigs to a cage. The number alive is indicated at weekly periods, the line dropping accordingly. The figure at the end of a line indicates the number of pigs alive at the time shown. The experiments were stopped soon after all pigs inoculated in ozone were dead. The figures in the top line indicate weeks of the experiment; those at the left of the charts indicate the numbers of the cages. In most of the experiments there were two

cages in each room, one as a check, the other containing pigs inoculated with tuberculosis. In charting it seemed to be desirable to group the check, or uninoculated, animals together at the top of the chart and the inoculated ones at the bottom. The average amount of ozone in the air of the room containing the cage is indicated near the beginning of the lines. Further explanations are made in connection with each chart.

ORDER OF PUBLICATION.

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD DAY.

WEDNESDAY—MORNING SESSION.

REPORT OF THE REPRESENTATIVES AT THE NATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH.

The eighth annual meeting of the National Conference on Pharmaceutical Research was held at Rapid City, S. Dak., August 24, 1929. Delegates were present from fourteen of the sixteen organizations entitled to representation. Delegates from several of the organizations that had no reports on progress in pharmaceutical research to make merely extended expressions of good will. Brief digests of a few of the more important reports from other representatives are given herewith:

SCIENTIFIC REPORTS.

S. L. Hilton stated that the age-old problem of enteric coatings was still before pharmacists and continued to be of great clinical importance. He said that both salol and keratin coatings had been demonstrated to be faulty, if not absolutely worthless, and that the formaldehyde-gelatine method developed by W. L. Scoville was far from perfect. If the gelatine capsules are immersed too long (more than 20 minutes) or the treated capsules are kept too long, the coating becomes insoluble in the intestinal fluid and the medication produces no results; if the immersion is for too short a time, the capsules dissolve in the stomach. Neither situation is desired by the clinician. In discussing the report, E. F. Cook called attention to the work on keratin done in the laboratory of the American Medical Association some years ago. This did not solve the problem of enteric coatings; on the contrary, it increased the difficulty of the situation.

E. F. Cook reported that recent work on sirup of wild cherry and tincture of aconite had shown both preparations to be unstable. He stated that the best keeping fluidextract of licorice was made by adding the ammonia water after the preparation had been almost completely manufactured and not by adding it to the menstruum before percolation.

In discussing the importance of bioassays, W. L. Scoville was of the opinion that the work on the chemical assays of drugs was in a satisfactory condition and that researches for the future should be directed toward making pharmaceuticals and galenicals more stable on keeping.

G. D. Beal emphasized the importance of the hydrogen-ion concentration factor in the stability of pharmaceuticals, both in manufacture and storage. He also spoke of the increasing importance of microchemical tests in many phases of qualitative analysis. The increasing use of aluminum ware in cookery renders the study of the question of the toxicity of aluminum in foods more and more urgent, he said.

H. W. Youngken quoted from several papers on the pharmacognosy of little used drugs, particularly on several species of viburnum. H. H. Rusby called attention to the importance in therapy of some little used drugs. He also mentioned the urgent need of depositing in permanent museums samples of all plant materials on which pharmaceutical research is being conducted. Only by so doing will confusion be avoided in the future, he stated.

E. Kremers pleaded for a chemical and pharmacological study of such drugs. E. N. Gathercoal mentioned a report on "Color Standardization", which he had prepared for presentation at the meeting of the American Pharmaceutical Association, calling attention to the large number of terms used in the U. S. Pharmacopeia to describe the colors of drugs or color reactions, many of which are confusing, indefinite or contradictory. He stated that effort is being made to prepare a list of terms which will convey better concepts of color, and that the officials of the Bureau of Standards and manufacturers of glassware and pharmaceuticals are cooperating in this study.

J. C. Krantz, Jr., described a book surveying the achievements in pharmacy which had been prepared under his direction as chairman of a committee. The book is called "Fighting Disease with Drugs". To date he has been unable to find either a publisher or a philanthropist willing to finance publication.

L. E. Warren reported on the researches in the methods for the analysis of drugs of the A. O. A. C. for the year. He mentioned that the published results filled about 73 pages of the journal of that association, and that satisfactory methods of analysis had been worked out for chloroform and carbon tetrachloride and for mercurous iodide and pilocarpine hydrochloride in tablets. Tentative microchemical tests for the identification of cinchonidine, cinchonine, quinidine and quinine have been adopted.

The most notable feature of the meeting was the adoption of a new constitution and by-laws. Probably the clause of the new constitution of most interest to this association is that which prescribes representation from constituent organizations. Each organization may send four

delegates to the annual meeting, but it is entitled to but one vote. Consequently, in naming its delegates the organization should designate one as chairman to cast the vote.

Officers elected were:

Chairman.....E. N. Gathercoal.
Vice-chairman.....E. F. Cook.
Secretary.....J. C. Krantz, Jr.
Treasurer.....P. H. Heisler.
Executive Committee.....H. V. Arny, L. E. Warren and L. L. Walton.

L. E. WARREN.
 M. R. THOMPSON.

Approved.

REPORT OF COMMITTEE ON EDITING METHODS OF ANALYSIS.

As many of the members are aware, the five-year period for the revision of *Methods of Analysis* comes to an end in 1930. An attempt to devise a plan for revision somewhat different from the former plan is contemplated. About the time of the last revision Mr. Doolittle, knowing that such a record would be of great assistance to the committee assigned to the work of revising the 1930 edition, compiled a report of all the changes made at the 1925 meeting. After Mr. Doolittle's death, R. W. Balcom, who was then Chairman of the Board of Editors of *The Journal*, compiled this report, and since Dr. Balcom's resignation from this position it has been done by the Associate Editor of *The Journal*. Therefore the work of the committee will be lessened to a slight degree, at least, since the reprints of these reports for the five years are available.

It is the conception of the secretary that it is necessary to appoint some one person who can devote most of his time to attending to this work and that he be assisted by a group or committee of advisers. This plan was agreed to by the Executive Committee at the meeting held Sunday night, and the following editors of *Methods of Analysis*, 1930 edition, were appointed by that committee and affirmed by the president: W. W. Skinner, chairman; J. A. LeClerc, J. W. Sale, L. E. Warren, G. G. Frary and Marian E. Lapp. Dr. LeClerc will be the executive officer of the committee and will critically review and assemble the data. It is hoped that the revised edition will be ready for distribution about January 1, 1931.

W. W. SKINNER.

Approved.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FORTY-FIFTH ANNUAL CONVENTION, OCTOBER 28-30, 1929¹.

I. FERTILIZERS.

(1) Under the word "Determination", sec. 10 (a), p. 3, the following sentence was inserted: "Not applicable in the presence of sulfates (first action)".

(2) The first line of sec. 9, p. 3, was revised to read as follows: "Treat 2 grams of the sample as directed under 6 (a), (b), (c), (d) or (g), etc." (first action).

(3) The Robertson method for the determination of nitrate nitrogen in mixed fertilizer containing cyanamide or urea was adopted as a tentative method to replace the present tentative Jones method².

The method is as follows:

Determine the total nitrogen as directed under 27 or 29, p. 9. Weigh out 2.0 grams of the fertilizer mixture and wash to 200 cc. with distilled water. Determine the nitrogen in the residue as directed under 19, 22 or 24. The difference between the total nitrogen and the nitrogen in the residue gives the water-soluble nitrogen.

Determine the ammoniacal nitrogen in 50 cc. of the filtrate as directed under 33.

Place another 50 cc. portion of the filtrate in a 500 cc. Kjeldahl flask, add 2 grams of ferrous sulfate and 20 cc. of sulfuric acid (sp. gr. 1.84). Digest over a hot flame until all the water is evaporated and white fumes appear. Add 0.65 gram of mercury or its equivalent of mercuric oxide and digest until the solution is clear. The nitrate nitrogen is thereby driven off. Complete the determination as directed under 24. A pinch of a mixture of zinc dust and granular zinc (20 mesh) should be added to each flask before distillation to prevent bumping. The difference between the nitrogen thus obtained and the water-soluble nitrogen gives the nitrate nitrogen.

The ammoniacal nitrogen plus nitrate nitrogen equals mineral nitrogen. The total nitrogen less the mineral nitrogen gives the organic nitrogen.

(4) The Jones modification of the Robertson method was appended to the Robertson method with the following explanation: Note: "Applicable when a determination of water-soluble nitrogen is not needed".

JONES MODIFICATION OF THE ROBERTSON METHOD.

(Note: Applicable when a determination of water-soluble nitrogen is not needed.)

Weigh 0.5 gram of the sample into a Kjeldahl flask. Add 50 cc. of water and rotate gently, then add 2 grams of ferrous sulfate and rotate. Add 20 cc. of sulfuric acid, sp. gr. 1.84. Digest over a hot flame. When the water is evaporated and white fumes appear, add 0.65 gram of mercury and complete the digestion as in the regular Kjeldahl method. Cool, dilute, and distil as usual. The nitrogen thus found subtracted from the total nitrogen represents the nitrate nitrogen.

(5) The following changes were made in par. 40, p. 12, and in the revision published in *This Journal*, 11, 33 and adopted finally in 1928,

¹ Compiled by Marian E. Lapp, Associate Editor. Unless otherwise stated, all references in this report are to *Methods of Analysis*, A. O. A. C., 1925.

² *This Journal*, 11, 32 (1928).

This Journal, 12, 33: Delete the revised instructions for the preparation of alkaline permanganate solution and substitute the following:

(a) *Stock solution of potassium permanganate*.—Dissolve 50 grams of potassium permanganate in a liter of water. Dissolve 0.5 gram of sodium oxalate in 300 cc. of water and 10 cc. of concentrated sulfuric acid. Heat to 70°–80°C. and titrate with the potassium permanganate solution, using a Mohr pipet or an all-glass buret to contain the permanganate solution. 235.89 divided by the result of the titrations in cc. gives the concentration of potassium permanganate in grams per liter. Adjust the concentration to 25 grams per liter. Store at a temperature above 15°C.

(b) *Stock solution of sodium hydroxide*.—Dissolve 300 grams of sodium hydroxide in 1 liter of water. Cool before using.

(c) *Alkaline permanganate solution*.—Mix equal quantities of the stock solutions (a) and (b) and add 10 cc. of water for each liter of solution that the mixture is calculated to make. Use this solution immediately, as it is unstable.

(6) The following paragraph was added as a third paragraph to par. 42, as revised (*This Journal*, 11, 34):

Previous to digestion with alkaline permanganate, the washed sample may be transferred from the filter to the flask by spreading the filter on a metal dish bent to form a trough that fits the palm of the hand, brushing the larger portion of the material into the flask with a spatula, and washing in the remainder with 20 cc. of water from a 20 cc. pipet or small wash bottle. Do not add more water before the digestion with alkaline permanganate, but, with this exception, proceed as with the transfer of the dried material. (First action.)

II. SOILS.

No additions, deletions or other changes.

III. AGRICULTURAL LIMING MATERIALS.

No additions, deletions or other changes.

IV. PLANTS.

(1) Par. 1, p. 39, Preparation of Sample—Official, was replaced by the following paragraphs, adopted tentatively:

DIRECTIONS FOR SAMPLING.

When more than one plant is sampled, include a sufficient number of plants to insure that the sample represents adequately the average composition of the entire lot of plants. The number of plants necessary for an accurate sample cannot be stated definitely; it will depend upon the variability in composition of the plants. Determine the details of the process by the purpose for which the sample is taken.

PREPARATION OF SAMPLE.

(1) *Mineral constituents*.—Remove all foreign matter from the material, especially adhering soil or sand. To prevent leaching, avoid excessive washing. Air dry as rapidly as possible to prevent decomposition or loss in weight by respiration, grind, and preserve in tightly stoppered bottles. If results are to be expressed on the fresh-weight

basis, record the weights of the sample before and after air-drying. When determinations of copper, manganese, zinc, iron and aluminum are made, take precautions to prevent contamination of the sample from dust during air-drying and from the grinding and sieving machinery.

The following directions for preparing samples of carbohydrates were adopted as tentative until further studies have been made:

(2) *Carbohydrates*.—After thoroughly removing all foreign matter, rapidly grind or chop the material into fine pieces. Add the weighed sample to sufficient hot, redistilled 95 per cent alcohol, to which sufficient precipitated calcium carbonate has been added to neutralize the acidity. Allowing for the water content of the sample, use sufficient alcohol to assure that the final concentration will be approximately 80 per cent. Heat, close to the boiling point, on a steam or water bath with frequent stirring for 30 minutes. Store the sample until needed.

(2) The methods for the determination of copper, manganese and zinc adopted as tentative last year¹ were adopted as official (first action).

V. INSECTICIDES AND FUNGICIDES.

The tentative method for the determination of Bordeaux-Paris green and Bordeaux-calcium arsenate, which was adopted as official (first action) last year, was adopted as official (final action).

VI. TANNING MATERIALS.

No additions, deletions, or other changes.

VII. LEATHERS.

No additions, deletions, or other changes.

VIII. WATERS, BRINE, AND SALT.

No additions, deletions, or other changes.

IX. FEEDING STUFFS.

The following electric air oven method for the determination of moisture in the absence of sugars, to be used when a vacuum oven is not available, was adopted as tentative:

Regulate an electric air oven to 135°C., $\pm 2^\circ$. Using low, covered aluminum dishes, weigh approximately 2 grams of the sample into each dish and shake until the contents are evenly distributed. With the covers removed, place the dishes and covers in the oven as quickly as possible and dry the samples for 2 hours. After placing the covers on the dishes, transfer them to a desiccator to cool and then weigh. Calculate the loss in weight as moisture.

¹ *This Journal*, 12, 35 (1929).

X. PRESERVATIVES AND ARTIFICIAL SWEETENERS.

The Monier-Williams method¹ for the estimation of added sulfurous acid, or added sulfite in food products was adopted as a tentative method.

XI. COLORING MATTER IN FOODS.

No additions, deletions, or other changes.

XII. METALS IN FOODS.

No additions, deletions, or other changes.

XIII. SUGARS AND SUGAR PRODUCTS.

(1) The following words were added to the official method for total ash, 106, p. 203: "taking precautions to guard against, or to correct for absorption of moisture during weighing" (first action).

(2) The following paragraph was inserted after sec. 109, p. 203:

ALKALINITY OF TOTAL ASH—OFFICIAL.

Add the alkalinities of the soluble and the insoluble portions, 108 and 109 (first action).

(3) The misprint in par. 112 (second), p. 204, was corrected to make the sentence read as follows: "Boil 280 grams of dry basic lead acetate [18 (C)] with 500 cc. of water".

(4) The tentative method for conductivity value, p. 205, par. 114, was revised by deleting the words: "and multiply by 10^{-6} ", and par. 115 was similarly revised by deleting the words: "and multiply by 10^6 ".

(5) The first three lines of the Meissl method, p. 195, and the table, p. 448, 10, were deleted (final action). First action was taken in 1927².

XIV. FRUITS AND FRUIT PRODUCTS.

No additions, deletions, or other changes.

XV. CANNED VEGETABLES.

No additions, deletions, or other changes.

XVI. CEREAL FOODS.

(1) In the tentative method (official first action) for sampling flour³ the word "steel", line 2, par. 3, was replaced by the word "metal".

(2) The Seidenberg method for the determination of chlorine in chlorine bleached flour⁴ was adopted as tentative.

¹ *This Journal*, 12, 120 (1929).

² *Ibid.*, 11, 36 (1928).

³ *Ibid.*, 9, 39 (1926).

⁴ *Ibid.*, 11, 132 (1928).

XVII. MEAT AND MEAT PRODUCTS.

The tentative method for the determination of added water in sausage and similar meat products¹ was revised to read as follows:

ADDED WATER IN SAUSAGE AND SIMILAR MEAT PRODUCTS.

Moisture.—Weigh accurately about 10 grams of the ground sample into a tared weighing bottle approximately 2 inches in diameter containing a short glass rod flattened at one end. Remove 2.5–3 grams for the protein determinations. Reweigh the remainder in the bottle, spreading it out in a thin layer over the sides and bottom by means of the glass rod, and use this sample for the determination of moisture. Dry in air at atmospheric pressure at a temperature of 101°–102°C. for approximately 16–18 hours, or until no significant loss of weight occurs on subsequent drying for a period of 2 hours. (If preferred, moisture may be determined according to paragraph 2, p. 237.)

Nitrogen.—Determine total nitrogen according to paragraph 17, 22, or 24, p. 6. Calculate the protein by multiplying the total nitrogen by the factor 6.25.

Added Water.—Multiply the percentage of protein calculated from the nitrogen determination by 4 and subtract the result from the percentage of moisture found. Report the difference, if any, as added water.

XVIII. GELATIN.

No additions, deletions, or other changes.

XIX. DAIRY PRODUCTS.

(1) The official Schmidt-Bondzynski method for the determination of fat in cheese (p. 279) was changed by substituting for the phrase “*** 0.5 gram of sand to prevent bumping * * *”, the phrase “* * * about 0.5 gram of sand, previously digested with concentrated hydrochloric acid * * *” (final action).

(2) The tentative method for the determination of fat in dried milk² was amended by adding the following: “After the first extraction with ether, add 4 cc. of 95 per cent alcohol to the liquid remaining in the extracting apparatus, mix, then proceed with the second extraction. In the third extraction add, if necessary, sufficient water to raise the level of the aqueous layer to its original volume”.

(3) The method proposed by Waterman³, modified by the substitution of the words, “filter clear, taking care to prevent evaporation during filtration”, for the present specific directions for filtration, was adopted as a tentative method.

XX. FATS AND OILS.

(1) The cold test for testing salad oils, other than olive⁴, was made official (first action).

¹ *This Journal*, 12, 43 (1929).

² *Ibid.*, 8, 482 (1925).

³ *Ibid.*, 10, 261 (1927).

⁴ *Ibid.*, 12, 46 (1929).

(2) The lead-salt-ether method for the determination of saturated and unsaturated fatty acids¹ was made official (final action).

(3) The combined Reichert-Meissl and Polenske method was made official and substituted for the present separate methods under "soluble" and "insoluble volatile acids" (pp. 290-291), with the exception that the illustration of apparatus on p. 292 was retained (first action).

The method follows:

REICHERT-MEISSEL AND POLENSKE VALUES.

REAGENTS.

(a) *Sodium hydroxide solution* (1 + 1).—Protect the solution from contact with carbon dioxide. Allow the solution to settle and use only the clear liquid.

(b) *Dilute sulfuric acid*.—Add 200 cc. of concentrated acid to 800 cc. of water.

(c) *Standard 0.1 N sodium hydroxide solution*.

(d) *Indicator*.—95 per cent by volume alcoholic solution of phenolphthalein.

(e) *Pumice stone*.—Heat small pieces to a white heat, plunge into water, and keep under water until used.

(f) *Glycerol soda solution*.—Add 20 cc. of the 1 + 1 sodium hydroxide solution to 180 cc. of pure concentrated glycerol.

DETERMINATION.

Weigh accurately 5 grams of the sample to be tested into a clean, dry, 300 cc. flask; add 20 cc. of the glycerol-soda solution, and heat over a flame or asbestos plate until complete saponification occurs, as shown by the mixture becoming perfectly clear. If foaming occurs, shake the flask gently. Add 135 cc. of recently boiled water, drop by drop at first to prevent foaming, then add 5 cc. of the dilute sulfuric acid and a few fragments of pumice stone. Distil without previously melting the fatty acids, using an apparatus of the exact dimensions illustrated in the diagram (p. 292). Rest the flask on a piece of asbestos board having a hole 5 cm. in diameter in the center, and so regulate the flame as to collect 110 cc. of the distillate in as near 30 minutes as possible and to allow the distillate to drip into the receiving flask at a temperature not higher than 18°-20°C.

When the distillation is complete, substitute for the receiving flask a 25 cc. cylinder to collect any drops that may fall after the flame has been removed. Mix without violent shaking, immerse the flask containing the distillate almost completely in water at 15°C. for 15 minutes, filter the 110 cc. of distillate through a dry filter paper 9 cm. in diameter, and titrate 100 cc. with the standard sodium hydroxide solution, using phenolphthalein as an indicator. The pink color should remain unchanged for 2 or 3 minutes. The Reichert-Meissl value is the number of cubic centimeters of 0.1 N sodium hydroxide solution used times 1.1, after this result is corrected for the figure obtained in a blank determination.

Remove the remainder of the soluble acids from the insoluble acids upon the filter paper by washing with three successive 15 cc. portions of water, previously passed through the condenser, the 25 cc. cylinder and the 110 cc. receiving flask. Then dissolve the insoluble acids by passing successive 15 cc. portions of neutral alcohol, 95 per cent by volume, through the filter paper, each portion having previously passed through the condenser, the 25 cc. cylinder, and the 110 cc. receiving flask. Titrate the combined alcoholic washings with the standard sodium hydroxide solution, using

¹ *This Journal*, 12, 44. (1929)

phenolphthalein as indicator. The Polenske value equals the number of cubic centimeters of alkali solution required for the titration.

NOTE.—Unless these directions are actually followed in every detail as described, satisfactory results cannot be obtained.

(4) The Kirschner method for the determination of volatile acids, the silver salts of which are soluble, using standard solutions of sodium, potassium, or barium hydroxide for the titration, was adopted as official (first action). The method follows:

KIRSCHNER VALUE¹.

REAGENTS.

- (a) 0.1 *N* barium hydroxide solution or 0.1 *N* sodium hydroxide solution.
- (b) Silver sulfate.
- (c) 95 per cent by volume alcoholic solution of phenolphthalein.
- (d) Sulfuric acid.—Add 25 cc. of acid to 1000 cc. of water.
- (e) Pumice stone.—Heat small pieces to a white heat, plunge into water, and keep under water until used.

DETERMINATION.

To 100 cc. of the Reichert-Meissl distillate, in a 200 cc. Erlenmeyer flask, add 6 drops of phenolphthalein solution and titrate to a very faint pink with a 0.1 *N* barium hydroxide solution. Add 0.3 gram of finely powdered silver sulfate. During the next hour shake the mixture frequently, then filter and transfer 100 cc. of the filtrate into a 300 cc. flask. Add 10 cc. of the diluted sulfuric acid, 35 cc. of water, and a piece of aluminum wire or several small pieces of pumice stone. Distil 110 cc. in about 20 minutes, using the Polenske apparatus (p. 292). Titrate 100 cc. of the distillate with 0.1 *N* barium hydroxide solution; make a blank determination; and after correcting the number of centimeters of alkali solution used, calculate the Kirschner value according to the following formula: $K = \frac{A \times 121 (100 + B)}{10,000}$, where *A* = the corrected

Kirschner titration, and *B* = the number of cc. of standard alkali solution to neutralize the 100 cc. Reichert-Meissl distillate.

Butter fat gives Kirschner values from 19 to 26; coconut oil gives an average of 1.9 and palm kernel oil 1.0, whereas the majority of other fats and oils give values from 0.1 to 0.2.

XXI. BAKING POWDERS AND BAKING CHEMICALS.

No additions, deletions, or other changes.

XXII. SPICES AND OTHER CONDIMENTS.

No additions, deletions, or other changes.

XXIII. VINEGARS.

(1) The tentative method for total reducing substances after inversion (p. 326) was amended by substituting the words "5 cc. of dilute hydro-

¹ *Z. Nahr. Genussm.*, 30, 205 (1905).

chloric acid, as directed on p. 186, 23(b) or as on p. 187, 23(c)", for the words, "2.5 cc. strong hydrochloric acid, as directed on p. 186, 23(b)".

(2) The tentative method for non-volatile reducing substances (sugar), 15 (p. 326), was amended by substituting the words "10 cc. of dilute hydrochloric acid as directed on p. 186, 23(b) or as on p. 187, 23(c)", for the words "5 cc. of strong hydrochloric acid as directed on p. 186, 23(b)".

XXIV. COFFEES.

No additions, deletions, or other changes.

XXV. TEAS.

No additions, deletions, or other changes.

XXVI. CACAO PRODUCTS.

The section "Examination of fat extracted from milk chocolate", of the tentative method for the detection of coconut and palm kernel oils in cacao butter and fat extracted from milk chocolate¹ was amended as follows:

Milk fat, if present in cacao butter subjected to this test, produces a turbidity less in intensity than that produced by the same percentage of coconut or palm kernel oil. For example, cacao butter containing 10 per cent, 15 per cent or 20 per cent milk fat produces, respectively, no opalescence, a faint opalescence or an opalescence. For this reason, when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply the percentage of lactose in the cacao product by 0.8, or the percentage of casein by 1.1, to obtain the percentage of milk fat in the product, and from this result calculate the percentage of milk fat in the total fat. If this percentage corresponds to 15 per cent or less, a blank of cacao butter containing 15 per cent milk fat may be used; otherwise make up a mixture of cacao butter and milk fat in the proportions indicated by the calculations.

Test the fat extracted from the sample under examination, as directed for the examination of cacao butter, but use the prepared mixture of cacao butter and milk fat instead of the pure cacao butter for the blank. If the fat being tested contains coconut oil or palm kernel oil, the last filtrate, when acidified, will be more turbid or milky than the blank.

XXVII. FLAVORING EXTRACTS.

(1) The present official method for the determination of citral in lemon and orange oils and/or extracts (p. 354-5) was dropped (first action).

(2) The method for the determination of citral in lemon and orange oils and/or extracts, published in *This Journal*, 12, 48 (1929) was adopted as official (final action).

¹ *This Journal*, 11, 45 (1928).

(3) The official Kleber method (p. 355) was removed from its place under the heading "Lemon and Orange Oils—Citral" and placed under the heading "Lemon and Orange Oils—Total Aldehydes" (first action).

XXVIII. WINES.

No additions, deletions, or other changes.

XXIX. DISTILLED LIQUORS.

No additions, deletions, or other changes.

XXX. BEERS.

No additions, deletions, or other changes.

XXXI. DRUGS.

(1) The following iodate method for the assay of mercuric iodide in tablets was adopted as tentative:

REAGENT.

Standard potassium iodate.—Dissolve 3 grams of pure potassium iodate, previously dried at about 120°C., in water and dilute to 1 liter. 1 cc. = 0.00637 gram of HgI_2 .

DETERMINATION.

Powder and mix a representative number of the tablets. Weigh accurately a sufficient quantity of the powdered material to represent 1-2 grains of mercuric iodide. Transfer to a 300 cc. glass-stoppered Erlenmeyer flask. To the flask add a cooled mixture of 30 cc. of concentrated hydrochloric acid, 20 cc. of water, and about 5 cc. of chloroform. Rotate the flask to disintegrate the powder and dissolve the mercuric iodide.

Titrate with the standard potassium iodate, adding the solution rapidly while rotating the flask. When the iodine which is liberated during the first stage of the reaction has disappeared from the solution, insert the stopper and shake vigorously for about 30 seconds. Continue the titration slowly, shaking thoroughly after each addition, until the iodine color just disappears from the chloroform, marking the end point.

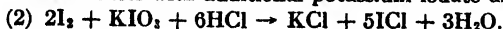
The following equation represents the completed reaction:



The reaction occurs in two stages, in the first of which iodine is liberated. This is represented as follows:



The iodine so liberated reacts with additional potassium iodate as follows:



(2) The following microchemical tests for brucine and caffeine were adopted as tentative:

MICROCHEMICAL TESTS FOR BRUCINE AND CAFFEINE.

REAGENTS.

Five per cent solutions of each of the following: Potassium iodide, mercuric chloride, and platinic chloride.

Krauf's reagent.—Dissolve 8 grams of bismuth nitrate in 20 cc. of nitric acid, sp. gr. 1.18. Dissolve 27.2 grams of potassium iodide in a little water. Mix the solution and dilute to 100 cc.

Millon's reagent.—Dissolve metallic mercury in an equal weight of strong nitric acid and dilute with an equal volume of water.

PREPARATION OF SAMPLES.

(1) *Controls*.—Dissolve 1 mg. of the pure alkaloidal salt in two drops of water to make an approximately 1-100 solution.

(2) *Alkaloids in compounds*.—Separate the alkaloid in pure form by extracting it from an ammoniacal solution with a suitable immiscible solvent and evaporate the solvent. To 1 mg. of the residue add, drop by drop, 0.1 *N* hydrochloric acid, avoiding an excess of acid, and dilute with water, if necessary, to approximately the same alkaloidal concentration as in (1).

(3) *Hypodermic tablets*.—Dissolve a portion of a tablet in water and dilute with water to approximately the same alkaloidal concentration as in (1).

IDENTIFICATION.

Place a drop of the alkaloidal solution on a clean glass slide; add a drop of reagent by means of a clean glass rod; and, without stirring or covering, examine under the microscope, using low power. (A magnification of 100-150 is suitable.) Note the kind of crystals formed and compare their characteristics with the descriptions given and also with a control.

Brucine and caffeine.

| ALKALOID | REAGENT | DESCRIPTION OF CRYSTALS |
|----------|-------------------|---|
| Brucine | Potassium iodide | Long masses of transparent rectangular plates, also rosettes of thin plates |
| | Mercuric chloride | Small dense rosettes |
| Caffeine | Mercuric chloride | Clusters of long radiating needle-shape crystals |

(3) The last part of the tentative method for the determination of atropine in tablets, p. 390, 5th line from end of method, was amended as follows:

Evaporate on the steam bath to about 5 cc. Add a measured excessive volume of 0.02 *N* sulfuric acid and continue the evaporation until the odor of chloroform has disappeared. Cool the solution and titrate back with 0.02 *N* sodium hydroxide, using 1 drop of methyl red test solution as indicator.

1 cc. of 0.02 *N* sulfuric acid = 5.784 mg. of atropine or 6.945 mg. of atropine sulfate.

(4) The following method for the determination of salicylic acid in the presence of other phenols was adopted as tentative:

REAGENTS.

(a) *U. S. P. ether*.

(b) *Dilute sulfuric acid* (1 + 9).

(c) *Saturated sodium bicarbonate solution*.

(d) *Chloroform-ether solvent*.—Mix 2 volumes of chloroform with 1 volume of ether.

(e) *0.1 N sodium hydroxide*.

(f) *Neutral alcohol*.

(g) *Dilute sodium hydroxide solution* (4 grams to 100 cc.).

PREPARATION OF SAMPLE.

Powders.—Weigh into a volumetric flask a sufficient quantity of the powder so that an aliquot of 25–50 cc. will contain approximately 0.13 gram of phenol. If acid, make alkaline with dilute sodium hydroxide and add 25 cc. in excess, fill to mark with water, and shake well.

Liquids.—For liquid samples proceed as under “Determination”.

DETERMINATION.

Transfer to a separatory funnel a sufficient quantity of solution to represent about 0.13 gram of phenol. Acidify with dilute sulfuric acid and extract with ether, using 20, 15, 15 and 10 cc. portions, respectively, until extraction is completed. Combine the ether in a separatory funnel, then shake with sodium bicarbonate solution, using 15, 15 and 10 cc. portions, respectively, and finally with 15 cc. of water. Combine the sodium bicarbonate solutions and the washing and extract the solution with 15 cc. of ether. Add the latter to the main bulk of ether and reserve for the phenol determination. Acidify the sodium bicarbonate solution with strong hydrochloric acid. Extract with chloroform-ether solvent, using 30, 25, 20 and 10 cc., respectively, until the salicylic acid is completely removed. Filter the solvent into a beaker through cotton previously saturated with chloroform. Evaporate to 5 cc. on a covered steam bath with the aid of an electric fan. Allow the last 5 cc. to evaporate spontaneously. Dissolve the residue in 10 cc. of neutral alcohol and titrate with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator.

1 cc. 0.1 *N* sodium hydroxide = 0.01381 gram of salicylic acid ($C_6H_4(OH)COOH$).

(5) The method published last year for the determination of total alkaloids in ephedra¹ was adopted as tentative, with the following alteration: Change the directions, “and shake the mixture intermittently for 2 hours; then allow to macerate 4 hours”, to read as follows: “allow to macerate at least 4 hours with occasional shaking”.

(6) The following method for the determination of toluol-insoluble material in rosin was adopted as tentative:

TOLUOL-INSOLUBLE MATERIAL IN ROSIN.

PREPARATION OF SAMPLE.

(1) If the sample is less than 200 grams, immediately before the determination is made powder it to pass a No. 10 sieve; mix thoroughly; and place in a wide-mouth bottle of such size that the sample completely fills it.

(2) If the sample is more than 200 grams, crush it to pass a $\frac{1}{8}$ -inch sieve; mix; quarter down to about 200 grams, and treat as described in (1).

PROCEDURE.

Place 50 grams of the freshly-powdered sample in a 300 cc. beaker; add 150 cc. of toluol, free from water and non-volatile residue; and dissolve the sample with the aid of heat and occasional shaking. When the solution is apparently complete (no particles of rosin visible), filter at once through a 25 cc. porcelain Gooch crucible which has been previously prepared with a mat of pure, well-washed asbestos (such as is used for the determination of barium sulfate) and which has been finally washed thoroughly with

¹ *This Journal*, 12, 291 (1929).

the solvent used, dry in a boiling-water oven for 30 minutes, cool in a desiccator, and weigh. If the rosin filtrate is not clear, return it through the Gooch crucible until it is clear, finally washing the residue and the outside of the crucible free from rosin with additional hot solvent. Dry the crucible and contents to constant weight at 105°-110°C. in an oven (1 hour usually suffices), cool in a desiccator, weigh, and calculate the percentage of toluol insoluble.

XXXII. REFERENCE TABLES.

No additions, deletions, or other changes.

EGGS AND EGG PRODUCTS.

(1) The tentative 98°C. vacuum-oven method for the determination of moisture¹ was adopted as official (first action).

(2) The tentative method for the determination of ash² was adopted as official (first action).

CAUSTIC POISONS.

The following modified Chapin method³ for the estimation of phenol in such products as cresol, saponified cresol solutions, coal-tar dips, disinfectants, fly sprays, and other similar preparations, was adopted as tentative:

PHENOL.

Modified Chapin Method—Tentative.

REAGENTS.

(a) *Dilute nitric acid.*—Blow air through strong nitric acid until it is colorless, then dilute 1 volume of this acid with 4 volumes of water.

(b) *Millon's reagent.*—Treat 2 cc. of mercury in a 200 cc. Erlenmeyer flask with 20 cc. of strong nitric acid. Place the flask under a hood, and after the first violent reaction is over shake vigorously to effect subdivision of the mercury and maintain action. After approximately 10 minutes, when the action has practically ceased even in the presence of undissolved mercury, add 35 cc. of water. If basic salt separates, add sufficient dilute nitric acid to dissolve it. Next add a 10 per cent solution of sodium hydroxide dropwise with thorough mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity. Then add 5 cc. of dilute nitric acid and mix well. Since the solution deteriorates do not use it later than the day following the day of preparation.

(c) *Standard phenol.*—Prepare a stock solution by dissolving a weighed quantity of the pure substance (congealing point not lower than 40°C.) in a sufficient quantity of water to make not less than a 1 per cent solution. From this stock solution make a 0.025 per cent solution in additional distilled water. (This second solution constitutes the final standard and it should be prepared on the day it is to be used.)

(d) *Dilute formaldehyde solution.*—Dilute 2 cc. of commercial 37 per cent formalin solution to 100 cc. with distilled water.

¹ *This Journal*, 9, 56 (1926).

² *Ibid.*, 12, 55 (1929).

³ U. S. Dept. Agr. Bull. 1308 (1924).

APPARATUS.

- (1) *Nessler cylinders*.—50 cc. tall form.
- (2) *Test tubes*.—About 180 mm. x 20 mm., provided with rubber stoppers and marked at 20 cc.
- (3) *Water bath for heating the test tubes*.—A beaker containing a disk of wire gauze raised slightly from the bottom may be used.

DETERMINATION.

Weigh by difference approximately 10 grams of sample into a separatory funnel (or use 10 cc. and calculate the weight from the density of the sample). Add 50 cc. of kerosene, and extract three times with 100 cc. portions of water. Filter the aqueous extracts through a wet filter into a 500 cc. volumetric flask, and make to volume with distilled water.

Transfer a 5 cc. aliquot of this solution to a 200 cc. volumetric flask shortly before the determination is to be carried out, dilute to about 50 cc., add one drop of methyl orange indicator solution and then dilute nitric acid until the solution is practically neutral, make to volume, and shake well.

Place 5 cc. of the diluted solution in each of two of the marked test tubes, and in each of two additional test tubes place 5 cc. of the standard phenol solution (C). Next flow 5 cc. of Millon's reagent (b) down the side of each tube, mix, and place the tubes in a bath of boiling water. Continue the boiling for exactly 30 minutes, cool immediately and thoroughly by immersion in a bath of cold water for at least 10 minutes, and add 5 cc. of dilute nitric acid (a) to each tube.

Mix well, add 3 cc. of dilute formaldehyde solution (d) to one of each pair of tubes, make all the tubes to the 25 cc. mark with water, stopper, shake well, and put aside to stand overnight. The next day the contents of the tubes to which formaldehyde was added will have faded to a yellow, while the others will possess orange or red tints.

Pipet 20 cc. from each of the two phenol tubes and transfer to 100 cc. volumetric flasks, treat each with 5 cc. of the dilute nitric acid, make to the mark, and mix. The red flask contains the "phenol standard", and the yellow flask the "phenol blank". Transfer these solutions to burets. Pipet 10 cc. of each sample solution into Nessler tubes. The orange or red one constitutes the "unknown" and the yellow one the "sample blank", and each Nessler tube must be distinctly marked to avoid confusion. Next add to the sample blank tube a measured quantity of phenol standard from its buret and add the same volume of phenol blank to the unknown, thoroughly agitate, and compare the colors. When the tubes have been brought to a match, each cc. of the phenol standard employed is equivalent to 1 per cent phenol if a portion of sample weighing exactly 10 grams was used.

REPORT OF THE BOARD OF EDITORS.

In line with the enlarged scope of the field covered by the Contributed Papers Section, which was brought to the attention of the members again last year, it is proposed to introduce a new feature in *The Journal*. Beginning with the February number of the ensuing year we will establish an Editorial Section, in which there will be presented editorials dealing with the trend of Agricultural Chemical Research as indicated by the more prominent papers in the respective fields covered by them.

It is apparent that the members are not taking full advantage of the space available for publication in the Contributed Papers Section, and we wish to stress again the policy presented to the association in our report of last year. We urge you to make use of this section and request that you let others know of the opportunity. Approximately the same number of pages have been contributed this year as appeared in 1928, but we confidently hope to double this next year. We feel that the quality of the papers accepted has improved and are glad to report that papers from foreign authors have come to us.

The books reviewed have been few owing to our inability to secure reviewers. In order to carry on this section we must have the cooperation of all our members. In this connection we should like to hear from those who are willing to review at least one book a year so that we may keep this department up to date. In writing the Chairman of the Board, please state what lines you are particularly interested in. It is our policy to supply the reviewer with a complimentary copy of the book he reviews for his personal library.

R. B. DEEMER.

H. R. KRAYBILL.

F. C. BLANCK.

W. F. HAND.

H. D. HASKINS.

Approved.

No report was given by the Chairman of the Committee on Quartz Plate Standardization and Normal Weight.

REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS.

For Final Adoption as Official.

1. MURIATE OF POTASH (COMMERCIAL POTASSIUM CHLORIDE).

Muriate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) chiefly as chloride.

2. SULFATE OF POTASH (COMMERCIAL POTASSIUM SULFATE).

Sulfate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) chiefly as sulfate, and not more than two and one-half per cent (2.5%) of chlorine.

3. GROUND STEAMED BONE.

Ground steamed bone is a product resulting from grinding animal bones that have been previously steamed under pressure.

4. GROUND RAW BONE.

Ground raw bone is a product resulting from drying and grinding animal bones that have not been previously steamed under pressure.

5. TANKAGE.

The term *tankage* (without qualification) shall be restricted to meat and bone *tankage* derived from the rendered, dried, and ground by-products from the slaughter of animals, or from carcasses of animals that have died otherwise than by slaughter.

6. FISH TANKAGE, FISH SCRAP, DRY GROUND FISH, FISH MEAL FERTILIZER GRADE.

Fish tankage, fish scrap, dry ground fish, fish meal fertilizer grade is the dried ground product derived from rendered or unrendered fish.

7. GARBAGE TANKAGE.

Garbage tankage is the rendered, dried, and ground product derived from waste household food materials.

8. CRUDE, INERT, OR SLOW-ACTING NITROGENOUS MATERIALS.

Crude, inert, or slow-acting nitrogenous materials are unprocessed organic substances relatively high in nitrogen but having a very low value as plant food and showing a low activity by both the alkaline and neutral permanganate methods (below fifty per cent (50%) and eighty per cent (80%) respectively).

9. HOOF AND HORN MEAL.

Hoof and horn meal is a product resulting from the processing, drying, and grinding of hoofs and horns.

10. SUPERPHOSPHATE.

Superphosphate is the product resulting from mixing rock phosphate and sulfuric acid and/or phosphoric acid. The grade should always be used as a prefix to the name. Example: 16% Superphosphate.

It is recommended that the use of the term "Acid Phosphate" be discontinued.

Amended Tentative Interpretation and Definition.

PROCESS TANKAGES.

Process tankages are the products made from crude inert nitrogenous materials by processing under steam pressure, with or without the use of acids, for the purpose of increasing the activity of the nitrogen.

These products shall be called "Process tankages" with or without further qualification.

Second Recommendation as Tentative.

1. ORDER OF TERMS.

The *order of terms* in mixed fertilizers shall be nitrogen first, phosphoric acid second, and potash third.

2. STATEMENT OF GUARANTEES.

The *statement of guarantees* of mixed fertilizers shall be given in whole numbers.

3. ACIDULATED FISH TANKAGE, ACIDULATED FISH SCRAP.

Acidulated fish tankage, acidulated fish scrap is the rendered product derived from fish and treated with sulfuric acid.

4. SIGNIFICANCE OF THE NAME OF A MATERIAL USED AS THE BRAND NAME OR PART OF THE BRAND NAME OF A MIXED FERTILIZER.

When the name of a material is used as a part of the brand name of a mixed fertilizer, as for example blood, bone or fish, the nitrogen or phosphoric acid shall be derived from

or supplied entirely by the material named. When the name of a material is used as a brand or as part of a brand and the nitrogen and phosphoric acid is not supplied by the material named, the word "brand" shall follow the name of the material. Example: "Fish Brand Fertilizer".

5. AMMONIATED SUPERPHOSPHATE.

Ammoniated superphosphate is a product containing superphosphate and/or dissolved bone and nitrogenous compounds, but without the addition of potash.

6. ACTIVATED SEWERAGE PRODUCTS.

Activated sewerage products are made from sewage freed from grit and coarse solids and aerated after being inoculated with microorganisms. The resulting flocculated organic matter is withdrawn from the tanks, filtered with or without the aid of coagulants, dried in rotary kilns, ground, and screened.

First Recommendation as Tentative.

1. SHEEP MANURE WOOL WASTE.

Sheep manure wool waste is the by-product from wool-carding establishments, consisting chiefly of sheep manure with seeds and wool fiber.

2. AVAILABLE PHOSPHORIC ACID.

Available phosphoric acid is the sum of the water-soluble and the citrate-soluble phosphoric acid.

3. PEAT.

Peat is partly decayed vegetable matter of natural occurrence and is composed chiefly of organic matter with some nitrogen of low activity.

4. CHARRED PEAT.

Charred peat is peat dried at such temperature as to cause partial decomposition.

5. SULFATE OF AMMONIA.

Sulfate of ammonia is a commercial product composed chiefly of ammonium sulfate and containing twenty and five-tenths per cent (20.5%) or more of nitrogen.

6. CYANAMIDE AND UREA NITROGEN.

Cyanamide and urea nitrogen shall be classified as synthetic non-proteid organic nitrogen.

7. DICALCIUM PHOSPHATE.

Dicalcium phosphate is a manufactured product consisting chiefly of phosphoric acid in the dicalcic form.

8. AGRICULTURAL LIME.

Agricultural lime. It is recommended that the use of the term agricultural lime be discontinued and that each specific lime product used as a soil amendment be defined.

9. HIGH CALCIUM LIME PRODUCTS.

High calcium lime products are those classes of liming materials containing not more than 4 per cent of their total oxides as magnesium oxide.

10. HIGH MAGNESIUM LIME PRODUCTS.

High magnesium lime products are those liming materials containing more than 25 per cent of their total oxides in the form of magnesium oxide.

11. QUICKLIME, BURNED LIME, CAUSTIC LIME, LUMP LIME, UNSLAKED LIME.

Quicklime, burned lime, caustic lime, lump lime, unslaked lime is commercial calcium oxide and magnesium oxide resulting from heating suitable carbonates until substantially all the carbon dioxide has been eliminated.

12. HYDRATED OR SLAKED LIME.

Hydrated or slaked lime is the product obtained by treating quicklime with sufficient water or steam to combine with its oxides.

13. AIR-SLAKED LIME.

Air-slaked lime is the product obtained by exposing caustic lime to the atmosphere, whereby it absorbs both moisture and carbon dioxide.

14. GROUND LIMESTONE.

Ground limestone is the product obtained by grinding calcitic or dolomitic limestone. Seventy-five per cent (75%) or more should pass a 100-mesh sieve. It should contain calcium and magnesium carbonates equivalent to not less than forty-five per cent (45%) of calcium oxide or the mixed oxides of calcium and magnesium.

15. GROUND SHELL LIME.

Ground shell lime is the product obtained by grinding the shells of mollusks. Seventy-five per cent (75%) or more should pass a 100-mesh sieve and should contain calcium and magnesium carbonates equivalent to not less than 40 per cent of calcium oxide or the mixed oxides of calcium and magnesium.

16. MARL, GROUND SHELL MARL.

Marl, ground shell marl is the product obtained by grinding natural deposits of shell marl. Seventy-five per cent (75%) or more should pass a 100-mesh sieve. It should contain calcium and magnesium carbonates equivalent to not less than 40 per cent of calcium oxide or the mixed oxides of calcium and magnesium.

17. WASTE LIME, BY-PRODUCT LIME.

Waste lime, by-product lime is any industrial waste or by-product containing calcium or calcium and magnesium in forms that will neutralize acids. It may be designated by the prefixation of the name of the industry or process by which it is produced, i. e., gas-house lime, tanners' lime, acetylene lime waste, limekiln ashes, etc.

18. CALCIUM SULFATE, GYPSUM.

Calcium sulfate, gypsum is a natural lime product consisting chiefly of calcium sulfate and is accompanied by varying quantities of impurities including about 20 per cent of water. It does not neutralize acids.

19. KAINIT.

Kainit. A potash salt containing 20 per cent of potash (K_2O) may be properly called kainit.

Proposed for Future Consideration.

1. HIGH ANALYSIS FERTILIZER.

A high analysis fertilizer is a commercial fertilizer containing thirty per cent (30%) or more of phosphoric acid, potash or nitrogen, alone or in combination.

2. SOIL AMENDMENT.

A *soil amendment* is any substance that is added to the soil for the purpose of improving its physical or chemical character or promoting the growth of crops, exclusive of commercial fertilizer and barnyard manure.

3. ROCK PHOSPHATE.

4. SOFT PHOSPHATE WITH COLLOIDAL CLAY.

5. AMMONIUM PHOSPHATE.

C. H. JONES.

H. D. HASKINS.

R. N. BRACKETT.

J. W. KELLOGG.

G. S. FRAPS.

Approved.

No report was given by the Committee on Revision of Methods of Soil Analysis.

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES.

The work submitted by the referees and their associates has been carefully reviewed by the committee, and the recommendations thereon are set forth in the report of Subcommittees A, B and C. The association is again indebted to these workers for their substantial contributions to the methods and for other papers of equal excellence.

Increasing activity is evidenced by the appointment for the coming year of one additional referee, on coffee, and eight associate referees as follows: high analysis fertilizers, carbohydrates in plants, forms of nitrogen in plants, solids in solution of sucrose and organic acids, sulfonal and trional, emetine; chloroform and carbon tetrachloride, and lead in foods.

Substantial progress has been made in the direction of making the presentation of papers on the floor of the convention of more interest and value by means of brief and concise summaries of work done, illustrated where possible by lantern slides. The departures from this plan are largely due to oversight or perhaps to the fact that the contributors may be presenting papers for the first time. More general appreciation of the fact that minute details of apparatus and technic and lengthy recitals of figures are lost upon the listener and destroy the force and effect of a paper will result in further improvement in this phase of the meetings.

E. M. BAILEY.

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By A. G. McCALL (Bureau of Chemistry and Soils, Washington, D. C.),
Chairman: R. N. BRACKETT, Acting Chairman:
and H. H. HANSON.

WATERS, BRINE AND SALT.

It is recommended that the referee study the method described in the report for this year, or similar methods, for the determination of boric acid in waters.

Approved.

TANNING MATERIALS AND LEATHERS.

No report was submitted.

INSECTICIDES AND FUNGICIDES.

It is recommended—

(1) That the method for the determination of copper in Bordeaux-Paris green¹ and Bordeaux-calcium arsenate, which was adopted as official first action last year², be adopted as official, final action.

Approved.

(2) That the methods for the determination of mercury in organic mercurial seed disinfectants be studied collaboratively during the coming year.

Approved.

FLUORINE COMPOUNDS.

It is recommended that studies of methods of analysis for the determination of fluorine compounds be continued.

Approved.

CAUSTIC POISONS.

It is recommended—

(1) That the modified Chapin method for the estimation of phenol in such products as cresol, saponified cresol solutions, coal-tar dips, disinfectants, fly sprays, and other similar preparations, described in the report of the referee this year, be adopted as a tentative method. (See p. 49.)

Approved.

(2) That further study be directed along the lines of developing methods for the estimation of "free or chemically unneutralized" acids, and especially of "free and chemically uncombined ammonia".

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 63, par. 87.

² *This Journal*, 12, 37 (1929).

SOILS AND LIMING MATERIALS.

REACTION VALUE OF SOILS.

No report was submitted.

The recommendation made last year on this subject is repeated.

Approved.

LIMING MATERIALS.

It is recommended—

(1) That in the next edition of *Methods of Analysis, A. O. A. C.*, provision be made to insure against the error introduced by sulfuretted hydrogen in the determination of carbonate (carbon dioxide).

Approved.

(2) That a study be made to determine the neutralizing possibilities of the calcium-silica combinations that occur in materials that are offered for sale as "soil amendments".

Approved.

LESS COMMON METALS IN SOILS.

It is recommended—

(1) That further collaborative study be given to the methods proposed by the associate referee for the determinations of copper, manganese and zinc in soils, and that consideration be given to the applicability of this procedure to the determinations of arsenic, iron, titanium, nickel and cobalt.

Approved.

(2) That the committee repeats the recommendations made last year relative to the determination of boron and of fluorine in soils.

Approved.

FEEDING STUFFS.

STOCK FEED ADULTERATION.

It is recommended that the study of the Sterling method for the determination of hoof meal¹ be continued.

Approved.

MINERAL MIXED FEEDS.

It is recommended—

(1) That the method prepared by the referee for the determination of lime in mineral feeds be not adopted this year, but that further study be made with the view to adopting it, or the acetic acid modification, next year, thus avoiding the adoption of two methods for the same determination.

Approved.

¹ *This Journal*, 12, 129 (1929).

(2) That further work on the determination of iodine in mineral feeds be carried on by the use of methods proposed for consideration.

Approved.

(3) That methods for the determination of iodine in organic-mineral mixtures be studied.

Approved.

DETERMINATION OF MOISTURE.

It is recommended that the electric air oven method described by the referee be adopted as tentative, to serve in cases where a vacuum oven is not available.

Approved.

SUGARS AND SUGAR PRODUCTS.

HONEY.

It is recommended that Nelson's modification¹ of the Fiehe test for the detection of artificial invert sugar in honey be studied collaboratively during the coming year.

Approved.

MAPLE PRODUCTS.

It is recommended—

(1) That the method of preparation of sample be studied with a view to attaining closer agreement in results for dry matter content.

Approved.

(2) That to the present official method for total ash, *Methods of Analysis*, p. 203, sec. 106, last line, be added the words: "taking precautions to guard against, or to correct for, absorption of moisture during weighing" (first action).

Approved.

(3) That the following paragraph be inserted after sec. 109, p. 203, *Methods of Analysis*: "Alkalinity of Total Ash—Official. Add the alkalinities of the soluble and the insoluble portions, secs. 108 and 109." (First action.)

Approved.

(4) That the misprint in Sec. 112 (second) p. 204, be corrected so that the first sentence will read, "Boil 280 grams of dry basic lead acetate [18 (c)] with 500 cc. of water".

Approved.

(5) That further collaborative study be made of the Canadian lead number method, as outlined by the referee.

Approved.

(6) That the present tentative method for conductivity value, *Methods of Analysis*, p. 205, sec. 114, be revised by deleting the words: "and

¹ *This Journal*, 12, 323 (1929).

multiply by 10^3 "; sec. 115 be similarly revised by deleting the words: "and multiply by 10^6 "; and that the method as so revised be further studied with a view to adoption as official next year.

Approved.

STARCH CONVERSION PRODUCTS.

No report was submitted.

The committee calls the attention of the General Referee on Sugars and Sugar Products to this subject and suggests that he make some recommendation for disposition of this topic next year.

Approved.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS.

No report was submitted.

The committee repeats the recommendations made last year on this subject.

Approved.

POLARISCOPIC METHODS.

It is recommended—

(1) That a fundamental study be made of the effect of the simultaneous presence of invert sugar and of amino compounds on the determination of sucrose by inversion methods. This should preferably be done in the form of an individual research project rather than a collaborative investigation. Collaborative work may follow if results warrant.

Approved.

(2) That the associate referee study the effect of lead clarification on the results of Clerget determinations in cane sugar products.

Approved.

CHEMICAL METHODS FOR REDUCING SUGARS.

It is recommended—

(1) That the first three lines of the Meissl method, p. 195, and the table, p. 448, 10, *Methods of Analysis*, be deleted (final action). At the 1927 meeting of this association it was recommended that Meissl's method for the determination of invert sugar be discarded. This method, with its accompanying table, is an almost exact duplicate of Munson and Walker's method for invert sugar. The only variation consists of the addition of cold water at the termination of the reduction. This duplication of methods serves no useful purpose, is confusing, and space consuming. A careful survey of modern literature has shown that the method is obsolete.

Approved.

(2) That the reducing power of invert sugar by Munson and Walker's method be studied with a view to corroborating or revising Munson and

Walker's tables, and that further experiments be made to determine the reducing power of levulose.

Approved.

(3) That the study of Nyns' selective method for levulose¹ be continued, and that the effect of aldohexoses and pentoses be determined.

Approved.

(4) That the iodine method for aldose sugars be studied, with particular reference to the modification devised by Slater and Acree².

Approved.

FERTILIZERS.

It is recommended that an associate referee be appointed to study the problem of high analysis fertilizers to determine what changes, if any, are needed in the present methods of the association to make them adaptable to those products.

PHOSPHORIC ACID.

It is recommended—

(1) That under the word "Determination", sec. 10 (a), p. 3, *Methods of Analysis*, the following be inserted: "Not applicable in the presence of sulfates" (first action).

Approved.

(2) That the first line, sec. 9, p. 3, be revised to read: "Treat 2 grams of the sample as directed under 6 (a), (b), (c), (d), or (g), etc." (first action).

NITROGEN.

It is recommended—

(1) That the Robertson method for the determination of nitrate nitrogen in mixed fertilizer containing cyanamide or urea be adopted as a tentative method to replace the present tentative Jones method³. (See p. 38.)

Approved.

(2) That the Jones modification of the Robertson method be appended to the Robertson method with the following explanatory note: "Applicable when a determination of water-soluble nitrogen is not needed". (See p. 38.)

Approved.

(3) That further study be made of the Robertson method for the purpose of further improvements.

Approved.

NITROGEN ACTIVITY METHODS IN FERTILIZERS.

It is recommended—

(1) That the following changes be made in *Methods of Analysis*, par.

¹ Bull. assoc. école sup. brasserie. Louvain, 25, 63 (1925); C. A., 19, 1236 (1925).

² Unpublished.

³ This Journal, 11, 32 (1928).

40, p. 12, and in the revision published in *This Journal*, 11: 33, and adopted finally in 1928, *This Journal*, 12: 33 (first action): Delete the revised instructions for the preparation of the alkaline permanganate solution and substitute the following:

(a) *Stock solution of potassium permanganate*.—Dissolve 50 grams of potassium permanganate in a liter of water. Dissolve 0.5 gram of sodium oxalate in 300 cc. of water and 10 cc. of concentrated sulfuric acid. Heat to 75°–80°C. and titrate with the potassium permanganate solution, using a Mohr pipet or an all-glass buret to contain the permanganate solution. 235.89 divided by the result of the titrations in cc. gives the concentration of potassium permanganate in grams per liter. Adjust the concentration to 25 grams per liter. Store at a temperature above 15°C. (b) *Stock solution of sodium hydroxide solution*.—Dissolve 300 grams of sodium hydroxide in a liter of water. Cool before using. (c) *Alkaline permanganate solution*.—Mix equal quantities of the stock solutions (a) and (b) and add 10 cc. of water for each liter of solution that the mixture is calculated to make. Use this solution immediately, as it is unstable.

Approved.

(2) That to par. 42 as revised in *This Journal*, 11: 34, the following paragraph be added as a third paragraph:

Previous to digestion with alkaline permanganate, the washed sample may be transferred from the filter to the flask by spreading the filter on a metal disk bent to form a trough that fits the palm of the hand, brushing the larger portion of the material into the flask with a spatula, and washing in the remainder with 20 cc. of water from a 20 cc. pipet or small wash bottle. Do not add more water before the digestion with alkaline permanganate, but with that exception proceed as with the transfer of the dried material. (First action.)

Approved.

POTASH.

It is recommended that the associate referee be given further time to work out details of the Fraps method in comparison with the regular official method before a collaborative study is undertaken.

Approved.

PLANTS.

It is recommended—

(1) That an associate referee be appointed to study methods for the determination of carbohydrates in plants.

Approved.

(2) That an associate referee be appointed to study methods for the determination of various forms of nitrogen in plants.

Approved.

(3) That study of methods for the determination of iron and aluminum in plants be continued.

Approved.

PREPARATION OF PLANT MATERIAL FOR ANALYSIS.

It is recommended that par. 1, Plants, p. 39, Preparation of Sample—Official, be replaced by the following tentative methods:

1. DIRECTIONS FOR SAMPLING¹.

When more than one plant is sampled, include a sufficient number of plants to insure that the sample represents adequately the average composition of the entire lot of plants sampled. The number of plants necessary for an accurate sample cannot be stated definitely; it will depend upon the variability in composition of the plants. Determine the details of the procedure by the purpose for which the sample is taken. (Adopted as official, first action.)

2. PREPARATION OF SAMPLE.

(1) *Mineral constituents*.—Thoroughly remove all foreign matter from the material, especially adhering soil or sand. To prevent leaching, avoid excessive washing. Air dry as rapidly as possible to prevent decomposition or loss in weight by respiration, grind, and preserve in tightly stoppered bottles. If the results are to be expressed on the fresh-weight basis, record the weights of the sample before and after air drying. When determinations of copper, manganese, zinc, iron and aluminum are made, take precautions to prevent contamination of the sample from dust during air drying and from the grinding and sieving machinery. (Adopted as official, first action.)

Approved.

It is suggested that the following method of preparation of sample for carbohydrates be studied further.

(2) *Carbohydrates*.—After thoroughly removing all foreign matter, grind or chop the material into fine pieces rapidly. Add the weighed sample to sufficient hot redistilled 95 per cent alcohol to which sufficient precipitated calcium carbonate has been added to neutralize the acidity. Allowing for the water content of the sample, use sufficient alcohol to assure that the final concentration will be approximately 80 per cent. Heat, close to boiling point, on a steam or water bath with frequent stirring for 30 minutes. Store the samples until ready for analysis.

Approved.

LESS COMMON METALS IN PLANTS.

It is recommended—

(1) That the methods for the determination of copper, manganese and zinc in plants be made official (first action).

Approved.

(2) That further attention be given to methods for the determination of iodine in soils, agricultural limestones, forage crops and foods.

Approved.

(3) That further study be made of the methods for determining chlorine and that a report be made at the next meeting. Chlorine determinations in tobacco are of considerable economic importance, and it is apparent from the few results thus far obtained by the bomb method that the present official method is subject to error.

Approved.

TOTAL CHLORINE IN PLANTS.

It is recommended—

(1) That collaborative work be done on a number of samples of plant

¹ *Bottlers Gazette*, 73, 44 (1922); *Proc. Am. Soc. Hort. Sci.*, 1927, p. 191

material including sirups, the open Carius digestion with the addition of solid potassium permanganate being used.

Approved.

(2) That additional work be done on the determination of chlorine in pure organic compounds by this method.

Approved.

PAINTS, PAINT MATERIALS AND VARNISHES.

No report was submitted.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By L. E. WARREN (Food, Drug and Insecticide Administration, Washington, D. C.), *Chairman*: H. C. LYTHGOE and A. G. MURRAY.

SPECIFIC GRAVITY AND ALCOHOL.

No report was submitted.

It is recommended that study of the subject be continued.

Approved.

SPICES AND OTHER CONDIMENTS.

Transferred to Subcommittee C.

NAVAL STORES.

ROSIN.

It is recommended that the method for the determination of toluol-insoluble material in rosin, submitted by the referee, be adopted as tentative. (See p. 48.)

Approved.

TURPENTINE.

It is recommended that study of the subject be continued.

The associate referee stated that the methods were satisfactory, but that he desired to keep the subject open for possible improvements in detail.

Approved.

DRUGS.

CRUDE DRUGS.

The associate referee calls attention to an unofficial variety of viburnum that grows in the southern states along the Atlantic coast, and gives a complete description of the plant as well as of the drug. The information is interesting, but is not and cannot be presented in such form as to constitute a method suitable for adoption by the association.

Since viburnum is not a particularly important drug it is not deemed desirable to devote further attention to the subject. It is believed that consideration of histological methods in the work of this association should be confined to the more important crude drugs.

Approved.

Since various species of aconite, which are not the U. S. P. variety, have been offered for entry as the official drug, it is recommended that it be studied with the view to formulating concise directions for distinguishing the official from allied species.

Approved.

RADIOACTIVITY IN DRUGS AND WATER.

It is recommended that the methods for the preparation of samples and for the determination of radioactivity, published in *This Journal*, 8, 531 and 10, 362, be further tested by collaborative work.

Approved.

LAXATIVES AND BITTER TONICS.

It is recommended that the work on these drugs be continued under the title "Emodin-bearing Drugs".

Approved.

MERCURIALS.

It is recommended—

(1) That the iodate method for the assay of mercuric iodide in tablets be adopted as tentative. (See p. 46.)

Approved.

(2) That methods for the examination of calomel ointment and mercuric oxide ointment be studied.

Approved.

MICROCHEMICAL METHODS FOR ALKALOIDS.

It is recommended—

(1) That the microchemical tests for brucine and caffeine be adopted as tentative. (See p. 46.)

Approved.

(2) That other important alkaloids be further studied with a view to including them in a scheme for identification by microchemical methods.

Approved.

CHLOROFORM.

It is recommended that the determination of chloroform in mixtures containing halides be studied with the view to supplementing the present tentative method for determining chloroform in drugs.

Approved.

TERPIN HYDRATE.

No report was submitted.

It is recommended that the study of analytical methods for the determination of terpin hydrate be continued.

Approved.

SANTONIN.

It is recommended that study of this subject be continued.

ATROPINE.

It is recommended that the last part of the tentative method for the determination of atropine in tablets¹ be amended to read:

Evaporate on the steam bath to about 5 cc. Add a measured excessive volume of 0.02 *N* sulfuric acid and continue the evaporation until the odor of chloroform has disappeared. Cool the solution and titrate back with 0.02 *N* sodium hydroxide, using 1 drop of methyl red test solution as indicator.

1 cc. of 0.02 *N* sulfuric acid = 5.784 mg. of atropine or 6.945 mg. of atropine sulfate.

ETHER.

It is recommended that this subject be continued.

Approved.

BIOASSAY OF DRUGS.

It is recommended—

(1) That no further work be done on pituitarium or on U. S. P. drugs for which bioassays are official.

Approved.

(2) That the standards for pituitary powder proposed by the associate referee be referred to the Committee on Revision of the Pharmacopeia of the United States.

Approved.

FLUIDEXTRACT OF GINGER.

No report was submitted.

It is recommended that no further work on this topic be done.

Approved.

EPHEDRA.

It is recommended—

(1) That the method for the determination of total alkaloids in ephedra², described by the associate referee in 1928, with the modifications suggested by the associate referee in 1929, be adopted as tentative.

Approved.

(2) That the methods for the assay of ephedra in pharmaceuticals be further studied.

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 390.

² *This Journal*, 12, 291 (1929).

THYMOL.

It is recommended that the determination of thymol in mixtures be studied collaboratively.

Approved.

MENTHOL.

It is recommended that the method proposed by the associate referee in 1928¹ be studied collaboratively.

Approved.

BROMIDES-CHLORIDES.

No report was submitted.

It is recommended—

(1) That the methods described by the associate referee² in 1928 for the determination of bromides in the presence of chlorides be subjected to collaborative study.

Approved.

(2) That the problem of the separation of the three halogens—chlorine, bromine and iodine—by chemical means be further studied.

Approved.

(3) That the applicability of the potentiometric methods for the determination of chlorides and bromides be studied.

Approved.

OIL OF CHENOPODIUM.

It is recommended that the Paget method for the assay of oil of chenopodium³ be studied collaboratively.

Approved.

SALICYLATES AND OTHER PHENOLS IN MIXTURES.

It is recommended that the method for the determination of salicylic acid in the presence of other phenols, described by the associate referee, be adopted as tentative (see p. 47).

Approved.

SMALL QUANTITIES OF IODINE IN MIXTURES.

No report was submitted.

It is recommended that the subject be continued.

Approved.

BISMUTH COMPOUNDS IN TABLETS.

It is recommended that the subject be further studied collaboratively.

Approved.

PHENOLSULFONATES.

It is recommended that the subject be further studied collaboratively.

Approved.

¹ *This Journal*, 12, 300 (1929).

² *Ibid.*, 302.

³ *Analyst*, 51, 170 (1925).

COLORIMETRIC METHODS FOR VITAMINS.

It is recommended that study of the subject be continued.

Approved.

BEERS, WINES AND DISTILLED LIQUORS.

No report was submitted.

It is recommended that study of the subject be continued.

Approved.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS
OF REFEREES.

By H. A. LEPPER (Food, Drug and Insecticide Administration, Washington, D. C.), *Chairman*: J. O. CLARKE and C. D. HOWARD.

DAIRY PRODUCTS.

It is recommended by the general referee—

(1) That there be deleted from *Methods of Analysis*, 1925, XIX, 37, the description of the 50 per cent, 9 gram, long-neck, 9 inch cream-test bottle and the 50 per cent, 18 gram, long-neck, 9 inch cream-test bottle.

The committee believes it to be desirable to postpone this action pending further consideration and recommends that the referee consult with the American Dairy Science Association and the American Public Health Association, both of which have accepted the method as at present written, to ascertain their attitude toward the proposed change with a view to continuing the desirable uniformity which now exists in the methods of the three associations. The referee concurs in this recommendation.

Approved.

MILK.

It is recommended—

(1) That the method presented by the associate referee on visible dirt in milk be further studied, and that the associate referee confer with other associations interested in this line of work whose methods are now uniform with those of this association, with a view to preserving the existing uniformity.

Approved.

BUTTER.

It is recommended that the studies outlined in 1927¹ be continued.

Approved.

CHEESE.

It is recommended—

(1) That methods for lactose and sucrose in cheese be further studied.

Approved.

¹ *This Journal*, 11, 75 (1928).

(2) That the first action of last year amending the wording of the official Schmidt-Bondzynski method for the determination of fat in cheese¹ be made official (final action).

Approved.

(3) That the tentative methods for tartaric acid and citric acid in cheese, as amended in 1928², be studied collaboratively.

Approved.

(4) That the referee consider the need for further work on the phosphorus pentoxide: calcium oxide ratios of processed cheese and of methods for the detection of preservatives, coloring matters, emulsifying agents, other than above referred to, or other added substances and recommend what studies be continued or be dropped.

Approved.

MALTED MILK.

It is recommended—

(1) That the study of methods for the determination of lactose and other sugars in malted milk be discontinued for the present.

Approved.

(2) That the associate referee submit the method for the microscopic identification of malted milk³ to collaborative study.

Approved.

(3) That methods for the determination of butter fat in malted milk be studied.

Approved.

DRIED MILK.

It is recommended—

(1) That the tentative method for the determination of fat in dried milk⁴ be amended by adding the following instructions: "After the first extraction with ether, add 4 cc. of 95 per cent alcohol to the liquid remaining in the extraction apparatus, mix, then proceed with the second extraction. In the third extraction add, if necessary, sufficient water to raise the level of the aqueous layer to its original volume".

Approved.

(2) That further study be made of the details of the tentative method for fat, including time and temperature of heating with ammonia, and avoidance of loss of fat during transfer to the extraction apparatus.

Approved.

(3) That study be made of the sampling of dried milks.

Approved.

¹ *This Journal*, 12, 44 (1929).

² *Ibid.*, 77.

³ *Ibid.*, 12, 238 (1929).

⁴ *Ibid.*, 8, 482 (1925).

ICE CREAM.

It is recommended—

(1) That work be discontinued on Recommendation (1) of last year under ice cream¹.

(2) That the associate referee consider the two other recommendations of last year and also recommend what further studies may be necessary.

MILK PROTEINS.

It is recommended that the method proposed by Waterman², modified by the substitution of the words, "filter clear, taking care to prevent evaporation during filtration", for the present specific directions for filtration be adopted tentatively and further studied collaboratively.

Approved.

QUALITATIVE TESTS.

It is recommended that the method for the determination of gelatin in milk be studied, especially in its application to evaporated milk.

Approved.

COFFEES.

It is recommended that the methods for caffeine in coffee be studied with respect to their applicability to coffees the caffeine content of which has been materially reduced.

Approved.

MEAT AND MEAT PRODUCTS.

It is recommended that the wording of the tentative method³ be revised (see p. 42) and that this method as well as other methods for the determination of moisture in meat and meat products be studied collaboratively.

Approved.

SEPARATION OF MEAT PROTEINS.

No report was submitted. The recommendations of last year are repeated.

Approved.

FATS AND OILS.

It is recommended—

(1) That the cold test for testing salad oils, other than olive⁴, be made official (first action).

Approved.

(2) That the lead-salt-ether method for the determination of saturated and unsaturated fatty acids⁵ be made official (final action).

Approved.

¹ *This Journal*, 12, 78 (1929).

² *Ibid.*, 10, 261 (1927).

³ *Ibid.*, 43.

⁴ *Ibid.*, 46.

⁵ *Ibid.*, 44.

(3) That the combined Reichert-Meissl and Polenske method¹ be made official and substituted for the present separate methods under "soluble" and "insoluble volatile acids"², with the exception that the illustration of apparatus on p. 292 be retained (first action).

Approved.

(4) That the Kirschner method³ using standard solutions of sodium, potassium or barium hydroxide for the titration, as described in the report of the referee, be made official (first action).

Approved.

(5) That methods for the determination of moisture in fats and oils be studied with particular reference to the rapid hot-plate procedure.

Approved.

(6) That methods for the determination of the hexabromide number (ether insoluble) of drying oils be studied.

Approved.

BAKING POWDERS AND BAKING CHEMICALS.

It is recommended—

(1) That the official gravimetric (Knorr)⁴ and the gasometric (Chittick)⁵ methods for carbon dioxide in baking powder be studied.

Approved.

(2) That further study be made of the tentative method⁶ for the determination of aluminum by precipitation with phenylhydrazine.

Approved.

(3) That study on the separation and determination of the different forms of phosphates used as baking acids be discontinued for the present.

Approved.

EGGS AND EGG PRODUCTS.

TOTAL SOLIDS, FAT, LIPOIDS, AND LIPOID PHOSPHORIC ACID (P_2O_5).

It is recommended—

(1) That methods for the determination of fat (acid hydrolysis), lipoids, and lipid phosphoric acid (P_2O_5) be studied collaboratively.

Approved.

(2) That methods for the determination of total phosphoric acid (P_2O_5) with consideration of the use of potassium hydroxide and magnesium acetate or nitrate as fixing agents be studied.

Approved.

(3) That methods for the determination of added sugars be studied.

Approved.

¹ *Ind. Eng. Chem.*, 18, 1346 (1926).

² *Methods of Analysis*, A. O. A. C., 1925, 25-30.

³ *Analyst*, 30, 205 (1905).

⁴ *Methods of Analysis*, A. O. A. C., 1925, 301.

⁵ *Ibid.*, 305.

⁶ *This Journal*, 12, 46 (1929).

(4) That the tentative 98°C. vacuum-oven method for the determination of moisture¹ be adopted as official (first action).

Approved.

(5) That the tentative 112°–117°C. air-oven method² be further studied collaboratively with a view to its adoption as an official method.

Approved.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER AND ASH.

It is recommended—

(1) That the tentative method for the determination of ash³ be adopted as official (first action).

Approved.

(2) That the tentative method for the determination of unsaponifiable matter⁴ be studied collaboratively with a view to its adoption as an official method.

Approved.

(3) That the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in egg products be further studied in conjunction with the same methods for alimentary pastes and flour. Consideration should be given to the use of 1.2 per cent sodium chloride solution for the extracting of the sample and of alumina cream for clarifying and collaborative work should be done if possible.

Approved.

DETECTION OF DECOMPOSITION.

It is recommended—

(1) That the method for the determination of acid-soluble phosphoric acid (P_2O_5) be further studied and be accompanied by collaborative work.

Approved.

(2) That a study be made of methods for determining ammonia nitrogen and reducing substances as dextrose.

Approved.

PRESERVATIVES.

It is recommended—

(1) That the Monier-Williams method⁵ for the estimation of added sulfurous acid or added sulfite in food products be adopted as a tentative method and that it be studied collaboratively with a view to its substitution a year hence for the present official method⁶.

Approved.

¹ *This Journal*, 9, 56 (1926).

² *Ibid.*, 57.

³ *Ibid.*, 12, 55 (1929).

⁴ *Ibid.*, 56.

⁵ Reports on Public Health and Medical Subjects, No. 43. Ministry of Health, London, 1927. *This Journal*, 12, 120 (1929).

⁶ *Methods of Analysis*, A. O. A. C., 1925, 135, 31.

(2) That search be continued for a method for detecting saccharin in food products which shall be more expeditious than the present official method and shall not involve the destruction of the saccharin (repeated from last year).

Approved.

(3) That the effort be continued to devise a method for the separation and determination of saccharin and sodium benzoate, respectively, when both are present in a food product (repeated from last year).

Approved.

(4) That an effort be made to formulate satisfactory methods for the detection and separation of hydrogen peroxide and other preservatives, as well as sweeteners (repeated from last year).

Approved.

COLORING MATTER IN FOODS.

It is recommended—

(1) That additional samples of mixtures of amaranth and tartrazine be submitted to collaborative study.

Approved.

(2) That study of the problem of quantitative separation and estimation of fast green F C F from light green S F yellowish be continued.

Approved.

(3) That work be undertaken to separate the recently adopted dyes (sunset yellow, ponceau SX and brilliant blue F C F) from the other permitted colors.

Approved.

(4) That additional work be done on the separation of yellow A B and yellow O B from other oil-soluble dyes.

Approved.

METALS IN FOODS.

It is recommended—

(1) That volumetric methods for the determination of arsenic in foods be studied collaboratively.

Approved.

(2) That the work on boron, copper and tin be continued.

Approved.

(3) That study be continued on methods for the determination of lead, especially as applicable to the determination of this element in spray residue.

Approved.

(4) That the work on zinc be postponed.

Approved.

FRUITS AND FRUIT PRODUCTS.

It is recommended—

(1) That the work on the determination of solids in solutions of sucrose

and organic acids, as for example in sweetened fruit juices and other fruit products, be continued.

Approved.

(2) That the investigation of methods for the determination of the major bases as well as chlorine in plant or fruit ashes be continued.

Approved.

(3) That collaborative study of methods for fruit acids be made.

Approved.

(4) That a study be begun on the effects of a definite hydrogen-ion concentration of the extraction medium on the alcohol precipitate, pectic acid and ash of fruits and fruit products.

Approved.

CANNED FOODS.

It is recommended that study be made of methods for the detection of spoilage in canned foods.

Approved.

CEREAL PRODUCTS.

FLOUR.

It is recommended—

(1) That in the tentative method (official first action) for sampling flour¹ the word "steel" (line 2, par. 3) be replaced by the word "metal", and that the method be further studied collaboratively.

Approved.

(2) That the associate referee continue the study of rapid methods of ashing flour, baked products and alimentary pastes, particularly regarding the effect of the rare earths as aids thereto and the influence of temperature, as well as the applicability of glycerol-alcohol mixture.

Approved.

(3) That the associate referee study the nature of the losses occurring when ash is fused.

Approved.

(4) That special studies be made by the associate referee of the method for the determination of unsaponifiable matter in the fat of flour² before subjecting it to collaborative study.

Approved.

(5) That special studies be undertaken regarding the tentative method of determining glutenin in flour³, pending which all collaborative work be suspended.

Approved.

(6) That for the determination of the hydrogen-ion concentration either the hydrogen electrode or the quinhydrone electrode be used.

Approved.

¹ *This Journal*, 9, 39 (1926).

² *Ibid.*, 10, 35 (1927); 11, 37 (1928).

³ *Ibid.*, 12, 39 (1929).

(7) That methods for the determination of the diastatic value of flour be studied.

Approved.

(8) That the Seidenberg method for the determination of chlorine in chlorine bleached flour¹ be adopted as tentative.

Approved.

(9) That attention be directed to the study of methods for the detection of bleaching of flour by benzoyl and other peroxides.

Approved.

(10) That further collaborative study be made of the tentative method (Rask)² for the determination of starch in flour, bread and alimentary paste by comparing it with the diastase method as modified by Hartmann and Hillig³.

Approved.

(11) That the proposal to substitute the factor 5.83 for the factor 5.7 for converting nitrogen of wheat into protein be laid on the table.

Approved.

(12) That the tentative method (official first action) for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol in flour⁴ be further investigated.

Approved.

(13) That the methods of analysis used by foreign government chemists for the testing of flour imported from this country be further studied, and that the tentative method for the determination of acidity in flour⁵ be compared with foreign methods wherein alcohol is used as the extractive medium.

Approved.

(14) That no further collaborative work be done with the present method for determining gasoline color value of flour.

Approved.

BAKED PRODUCTS.

It is recommended—

(1) That collaborative study be made of the tentative method for the sampling of bread⁶ to determine the possibility of utilizing only one-half the loaf instead of the whole loaf and that different types of bread be tried.

Approved.

(2) That the tentative method (official first action) for the determination of total solids in an entire loaf of bread⁶ be studied collaboratively and include studies to determine the possibility of estimating the total

¹ *This Journal*, 11, 132 (1928).

² *Ibid.*, 37.

³ *Ibid.*, 9, 482 (1926).

⁴ *Ibid.*, 12, 40 (1929).

⁵ *Methods of Analysis*, A. O. A. C., 1925, 225.

⁶ *This Journal*, 9, 42 (1926).

solids in bread by utilizing only one-half of the loaf and that experiments be made with different types of bread.

Approved.

(3) That the tentative method for the determination of fat in bread by acid hydrolysis¹ be subjected to further study.

Approved.

(4) That further study be made of the methods of determining lipoids in baked products.

Approved.

(5) That special studies be made of the tentative method² now applicable for flour for the determination of unsaponifiable matter in the fat of bread and other baked products.

Approved.

(6) That consideration be given to the development of methods for the determination of milk solids in bread.

Approved.

(7) That consideration be given to the development of methods for the determination of rye in rye bread.

Approved.

(8) That the tentative method (official first action) for the determination of chlorides in baked products³ be studied collaboratively.

Approved.

(9) That the tentative method (official first action) for the determination of moisture in baked products⁴ be studied collaboratively.

Approved.

(10) That the tentative method (official first action) for the determination of crude fiber in baked products⁵ be studied collaboratively.

Approved.

(11) That the tentative method (official first action) for the determination of organic and ammoniacal nitrogen⁶ in baked products be studied collaboratively.

Approved.

(12) That further study supplemented by collaborative work be carried on with the tentative method of making an experimental baking test⁷.

Approved.

(13) That the associate referee make a record next year on the subject of total solids in an entire loaf of bread by the 130°C. air-oven⁸ and other rapid methods.

Approved.

¹ *This Journal*, 9, 41 (1926).

² *Ibid.*, 8, 441 (1925); 10, 33 (1927).

³ *Methods of Analysis*, A. O. A. C., 1925, 232; *This Journal*, 12, 40 (1929).

⁴ *Ibid.*, 9, 39 (1926); 12, 40 (1929).

⁵ *Methods of Analysis*, A. O. A. C., 1925, 225; *This Journal*, 12, 41 (1929).

⁶ *Methods of Analysis*, A. O. A. C., 1925, 232.

⁷ *This Journal*, 12, 41 (1929).

⁸ *Ibid.*, 9, 40 (1926).

(14) That consideration be given to the study of other baked products than bread.

Approved.

ALIMENTARY PASTES.

It is recommended—

(1) That the tentative method for collecting and preparing a sample of alimentary paste for analysis¹ be further studied with a view to making it official.

Approved.

(2) That the tentative method (official first action) for the determination of moisture in alimentary paste² be further studied.

Approved.

(3) That further study be conducted with the tentative F. A. C. method³ for the determination of the unsaponifiable matter in the fat of alimentary paste before submitting it to collaborative work.

Approved.

(4) That the tentative method (official first action) for water-soluble protein-nitrogen precipitable by 40 per cent alcohol⁴ be further investigated.

Approved.

(5) That the tentative methods (official first action) for determining total solids⁵, fat by acid hydrolysis⁶, and lipoids and lipid phosphoric acid (P_2O_5)⁷ in alimentary pastes be studied collaboratively.

Approved.

(6) That the official method for the determination of crude fiber in flour⁸ be studied collaboratively for adaptation of this determination to alimentary paste.

Approved.

VINEGARS.

It is recommended—

(1) That methods for total and soluble ash be further studied with particular attention given to the use of sucrose or other substances for reducing the time of heating and to the temperature of ashing.

Approved.

(2) That the methods for the determination of phosphoric acid be further studied in connection with studies on ash.

Approved.

(3) That the method for glycerol be further studied.

Approved.

¹ *This Journal*, 9, 43 (1926).

² *Ibid.*, 43; 12, 43 (1929).

³ *Ibid.*, 9, 45 (1926); 10, 35 (1927); 11, 39 (1928).

⁴ *Ibid.*, 12, 40, 43 (1929)

⁵ *Ibid.*, 9, 43 (1926)

⁶ *Ibid.*, 11, 38 (1928).

⁷ *Ibid.*, 9, 40 (1926).

⁸ *Methods of Analysis*, A. O. A. C., 1925, 225.

(4) That the tentative method for sulfates¹ be submitted to collaborative study with a view to its adoption as official.

Approved.

(5) That the method for polarization¹ be further studied, and that a variety of vinegars including malt, sugar and corn-sugar vinegars be used in the study.

Approved.

(6) That the tentative method for total reducing substances after inversion² be amended by substituting the words "5 cc. of dilute hydrochloric acid, as directed on p. 186, 23 (b) or as on p. 187, 23 (c)" for the words "2.5 cc. strong hydrochloric acid as directed on p. 186, 23 (b)". (Change in a tentative method to make it in harmony with methods for inversion.)

Approved.

(7) That the tentative method for non-volatile reducing substances (sugar)², 15, be amended by substituting the words "10 cc. of dilute hydrochloric acid as directed on p. 186, 23 (b) or as on p. 187, 23 (c)", for the words "5 cc. of strong hydrochloric acid as directed on p. 186, 23 (b)".

Approved.

(8) That the official method for total solids³ be studied, especially with reference to its application to vinegars high in solids, such as malt vinegars⁴.

FLAVORS AND NON-ALCOHOLIC BEVERAGES.

It is recommended—

(1) That the present official method for the determination of citral in lemon and orange oils and/or extracts⁵ be dropped (final action).

Approved.

(2) That the method for the determination of citral in lemon and orange oils and/or extracts published in *This Journal*, 12, 48-9 (1929) be adopted as official (final action).

Approved.

(3) That the official Kleber method⁶ be removed from its place under the heading "Lemon and Orange Oils—Citral" and placed under the heading "Lemon and Orange Oils—Total Aldehydes" (first action).

Approved.

(4) That more extensive collaborative work be done on the gravimetric method for the determination of total aldehydes in orange and lemon oils and/or extracts, described in this year's report of the referee,

¹ *Methods of Analysis*, A. O. A. C., 1925, 329.

² *Ibid.*, 326.

³ *Ibid.*, 325.

⁴ *This Journal*, 10, 520 (1927).

⁵ *Methods of Analysis*, A. O. A. C., 1925, 354-5.

⁶ *Ibid.*, 355.

or modification of it, and that the search be continued for other methods that are applicable to both oils and extracts.

Approved.

(5) That the study of the steam distillation method for the analysis of non-alcoholic flavors be discontinued for the present.

Approved.

GELATIN.

It is recommended—

(1) That further comparative study be made of the ashing and hydrolysis methods for the determination of copper and zinc¹.

Approved.

(2) That study of the preparation of samples be continued.

Approved.

CACAO PRODUCTS.

It is recommended—

(1) That the method proposed by the associate referee for the determination of crude fiber in bitter and sweet chocolate be studied further and that collaborative work be done.

Approved.

(2) That the proposed method for the determination of crude fiber be studied with a view to making it applicable to milk chocolate.

Approved.

(3) That the study of methods for the detection of foreign fats in cacao products be continued.

Approved.

(4) That the section, "Examination of fat extracted from milk chocolate", of the tentative method for the detection of coconut and palm kernel oils in cacao butter and fat extracted from milk chocolate² be amended as directed by the associate referee.

Approved.

(5) That methods for the determination of milk solids and sucrose in cacao products be studied.

Approved.

SPICES AND OTHER CONDIMENTS.

It is recommended—

(1) That the study of the lecithin phosphoric acid determination be continued and that consideration be given to the methods for lipoid P_2O_5 now used on eggs and egg products and alimentary pastes.

Approved.

(2) That the method proposed by the referee for reducing sugars before inversion be studied collaboratively, along with the present tentative methods for total solids, oil, reducing sugars after inversion and total acid in salad dressings³.

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 258.

² *This Journal*, 11, 45 (1928).

³ *Methods of Analysis*, A. O. A. C., 1925, 321-22.

REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL.

In the report that was presented last year data were given as to the history, organization, policy and nature of the work undertaken by the Crop Protection Institute.

Owing to changes and sickness, neither of your representatives was able to attend the last annual meeting of the Institute. Close contact with the work through the year has been maintained by correspondence.

The annual report of the chairman of the Board of Governors shows that substantial progress was made in the investigations under way and that the results obtained were satisfactory. This reflects credit both on the project committees and men performing the work. The fact that the work conducted under the auspices of the Institute is being financed by commercial interests proves that it is supplying a need and yielding worthwhile results.

The following projects are in progress at present:

- Crown Gall
- Volck Oil as an Insecticide
- Oxidized Oils as Insecticides
- Incorporation of Fungicides in Oxidized Oils
- Kopper's Sulfur
- Horticultural Tape
- Makepeace Project
- Monsanto Project
- Oil Sprays
- Horticultural Sprays
- Fungicides in Oil Sprays
- Pyrethrum Culture
- Petroleum Oil Insecticides
- Shale Oils
- Clymol Chemicals, Inc.
- Furfural Project

In addition to these projects the Institute continues to serve as a means for doing preliminary testing of promising materials.

The Institute is in good financial condition. It met all expenses and set aside \$1000 as a reserve fund.

H. J. PATTERSON.
W. H. MACINTIRE.

Approved.

REPORT OF SECRETARY-TREASURER.

The following resignations were received during the year:

D. H. Tilden, Associate Referee on Ash in Fruit Products and also Associate Referee on Chlorine in Plants.

E. L. Tague, Associate Referee on Diastatic Value of Flour.

M. F. Mason, Michigan State College, was appointed in Tilden's place, and Arnold Johnson replaced E. L. Tague.

It was impossible to find anyone able to take the Associate Refereeship on Reaction Value of Alkaline Soils, or that of Starch Conversion Products.

Since the publication of the list of referees and associate referees in No. 1 of Volume 12 of *The Journal*, the following additional appointments were made:

Arsenic: W. C. Taber, Food, Drug and Insecticide Administration, San Francisco, Calif.

Boron: O. F. Krumboltz, Bureau of Chemistry and Soils, Washington, D. C.

Tin: Urner Liddel, Bureau of Chemistry and Soils, Washington, D. C.

Copper and zinc: Reed Walker, Bureau of Chemistry and Soils, Washington, D. C.

Foreign Methods for Testing Flour: C. H. Bailey, University Farm, St. Paul, Minn.

Organic and ammoniacal nitrogen in air-dried baked cereal products: S. C. Rowe, Food, Drug and Insecticide Adm., Washington, D. C.

Crude fiber in alimentary pastes and in air-dried baked cereal products: W. F. Sterling, Food, Drug and Insecticide Adm., Washington, D. C.

Collecting and preparing samples of alimentary paste for analysis: J. B. Reed, Health Department, Washington, D. C.

Phenolsulfonate: Maurice Harris, Food, Drug and Insecticide Adm., Chicago, Ill.

W. H. MacIntire was appointed as a representative on the Board of Governors of the Crop Protection Institute of the National Research Council in place of B. L. Hartwell, resigned.

L. E. Warren and M. R. Thompson represented this association at the eighth annual meeting of the National Conference on Pharmaceutical Research.

It is with deep sorrow that the loss of four members of the association is recorded.

Anthony McGill, one of the Canadian members of the association, passed away on December 29, 1928. An obituary by G. E. Grattan was published in the August number of *The Journal*.

John K. Haywood died on November 3, 1928, at Emergency Hospital, Washington, D. C. The secretary prepared the obituary published in Number 2 of Volume 12.

More recently, on October 17th, occurred the death of one of the active members, R. W. Balcom. His passing was a distinct shock to his associates, since it was thought that he was in the best of health. A suitable obituary will be prepared by one of the members of the association.

Edwin Le Fevre also passed away suddenly last week while at his office. While not actively engaged in the work of the association, Dr. Le Fevre often attended the meetings.

Many letters requesting information on specific subjects have been received during the year and referred to the proper referees.

It may be well at this time to present the decision of the Executive Committee in regard to the handling of the editorial work of the association. Perhaps without your realizing it, and I think without full realization on the part of the officers, the editorial business of the association has increased considerably in the last few years. We now have three editorial boards, the Board of Editors for *The Journal*, the Board of Editors for *Methods of Analysis* and a Board of Editors of the two volumes of "Principles and Practice of Agricultural Analysis", which are being revised. Two years ago the Executive Committee designated Dr. C. A. Browne and myself as editor and associate editor of these books, authorizing us to select such collaborators as we thought advisable. Now I am sure it will require no argument to convince you that with three editorial boards it is very essential that there shall be close coordination and collaboration. There has been some coordination, of course, but it was not from any definite plan. I had expected at the meeting last year to present a program which would provide for a new set up of editorial organization. However, due to illness which confined me in the hospital almost up to the date of last year's meeting, the presentation of the plan was delayed. It was presented at the meeting of the Executive Committee Sunday evening. It provides for one committee which will be composed of the three editorial boards, and it will be known as the Editorial Committee of the Association. It will function under the Secretary-Treasurer as the coordinating officer and chairman of the committee. The finances of two groups, *Methods of Analysis* and *The Journal*, have already been coordinated in the office of the Treasurer. The revision of *Methods of Analysis* is going to involve a considerable amount of money and a large number of collaborators. The Executive Committee approved the plan, so that there is created in the association an Editorial Committee to decide on matters of policy, matters of finance, etc.

The vacancy on the Board of Editors on *The Journal* caused by the retirement of H. D. Haskins was filled by the committee on the concurrence of the President, by W. S. Frisbie.

I have a communication addressed to the secretary and signed by Dr. C. H. Jones of Vermont, our esteemed fellow member, which will be received with a great deal of regret.

Dear Dr. Skinner:

I hereby tender my resignation as Chairman of the Committee on Definitions of Terms and Interpretation of Results on Fertilizers. My physical condition has been

RECEIPTS—METHODS OF ANALYSIS AND JOURNAL

| <i>Methods of Analysis.</i> | | |
|-------------------------------|------------|------------------|
| Number | Price each | |
| 23 | \$5.50 | \$126.50 |
| 285 | 5.00 | 1,425.00 |
| 67 | 4.40 | 294.80 |
| 215 | 4.00 | 860.00 |
| 1 | 4.50 | 4.50 |
| 1 | 3.00 | 3.00 |
| | | <hr/> \$2,713.80 |
| Plus gain on exchange..... | | .25 |
| Total..... | | \$2,714.05 |
| Minus re-deposited check..... | | 5.00 |
| | | <hr/> \$2,709.05 |

| <i>Journal Subscriptions.</i> | | |
|--------------------------------|------------|------------------|
| Number | Price each | |
| 51 | \$5.50 | \$280.50 |
| 414 | 5.00 | 2,070.00 |
| 11 | 4.50 | 49.50 |
| 112 | 4.40 | 492.80 |
| 227 | 4.00 | 908.00 |
| 4 | 3.75 | 15.00 |
| 6 | 2.50 | 15.00 |
| 3 | 2.00 | 6.00 |
| 15 | 1.50 | 22.50 |
| 10 | 1.25 | 12.50 |
| | | <hr/> \$3,871.80 |
| Plus gain on exchange..... | | .58 |
| Total..... | | \$3,872.38 |
| Minus charge for exchange..... | | \$1.13 |
| Minus re-deposited checks..... | | 9.40 |
| | | <hr/> \$3,861.85 |

| <i>Advertisements.</i> | | |
|------------------------|------------|----------|
| Number | Price each | |
| 3 | \$15.00 | \$45.00 |
| 10 | 25.00 | 250.00 |
| | | <hr/> |
| Total..... | | \$295 00 |

| <i>Miscellaneous.</i> | | |
|--|--|--------|
| Refund for book, "Physics and Biochemistry of Bacteria"..... | | \$5.62 |
| Interest from bankruptcy account..... | | .33 |
| Rebate on fire insurance..... | | .19 |
| | | <hr/> |
| Total..... | | \$6.14 |

| <i>Reprints.</i> | | |
|---|--|--------|
| Agricultural Experiment Station, New Brunswick, N. J..... | | \$5.92 |
| C. A. Browne, Washington, D. C..... | | 4.00 |
| E. M. Emmert, Lexington, Ky..... | | 2.30 |
| D. T. Englis and V. C. Mills, Urbana, Ill..... | | 1.21 |
| R. Hertwig, Buffalo, N. Y..... | | 2.12 |
| William F. Kunke, Chicago, Ill..... | | 12.28 |
| W. H. MacIntire, Knoxville, Tenn..... | | 2.00 |
| J. S. McHargue, Lexington, Ky..... | | 6.21 |
| W. T. McClosky, Washington, D. C..... | | 1.50 |
| J. C. Munch, Baltimore, Md..... | | 4.50 |
| E. K. Nelson, Washington, D. C..... | | 2.00 |
| Experiment General, Kingston, R. I..... | | 6.70 |
| Arthur H. Thomas Co., Philadelphia, Pa..... | | 7.50 |
| F. W. Zeran, New York City..... | | 19.29 |
| J. C. Munch, Baltimore, Md..... | | 13.14 |
| M. J. Bliah, Lincoln, Neb..... | | 4.23 |
| S. Alfend, St. Louis, Mo..... | | 6.32 |
| New Hampshire State Laboratory, Concord, N. H..... | | 3.32 |
| H. Runkel, Chicago, Ill..... | | 4.92 |
| Purdue University, Lafayette, Ind..... | | 22.44 |
| A. R. Bliss, Memphis, Tenn..... | | 9.56 |
| R. J. McNeil, Philadelphia, Pa..... | | 9.25 |
| University of Minnesota, Minneapolis, Minn..... | | 6.38 |
| Max Phillips, Washington, D. C..... | | 5.00 |
| G. S. Jamieson, Washington, D. C..... | | 2.00 |
| | | <hr/> |
| Total..... | | 164.09 |

| | | |
|---|--|------------|
| Total for <i>Methods, Journal, Ads., Reprints and Miscellaneous</i> | | \$7,036.13 |
| Plus foreign collections..... | | 31.22 |
| Bank balance of October 1, 1928..... | | 787.99 |
| | | <hr/> |
| Total..... | | \$7,855.34 |

DISBURSEMENTS.

| | | Amount | Check No. |
|-------------|---|------------|-----------|
| 1928 | | | |
| Oct. 23 | Ace Letter Service, <i>Methods</i> and <i>Journal</i> billheads..... | \$13.65 | 273 |
| Oct. 23 | Industrial Printing Co., labels for <i>Methods</i> | 14.75 | 274 |
| Oct. 24 | Marie-Alice Bates, office expenses..... | 50.00 | 275 |
| Nov. 19 | A. M. Peter, back numbers of <i>Journal</i> | 4.00 | 276 |
| Nov. 27 | Postmaster, Washington, D. C., mailing <i>Journals</i> | 25.00 | 277 |
| Nov. 28 | J. D. Turner, back numbers of <i>Journal</i> | 12.00 | 278 |
| Nov. 29 | Industrial Printing Co., bill of 8-17-28..... | 1,282.15 | 279 |
| Dec. 4 | Industrial Printing Co., bill of 11-27-28..... | 3.50 | 280 |
| Dec. 10 | Industrial Printing Co., bill of 9-29-28..... | 105.75 | 281 |
| Dec. 21 | Postmaster, Washington, D. C., box rent, quarter ending 12-31-28..... | 2.00 | 282 |
| Dec. 27 | Marie-Alice Bates, office expenses..... | 50.00 | 283 |
| 1929 | | | |
| Jan. 4 | J. J. Betton, bond for M. A. Bates..... | 2.50 | 284 |
| Feb. 16 | Marie-Alice Bates, office expenses..... | 50.00 | 285 |
| Mar. 11 | Cash, refund to association account, dues of Minnesota. . | 5.00 | 286 |
| Mar. 22 | Postmaster, Washington, D. C., box rent, quarter ending 3-22-29..... | 2.00 | 287 |
| Mar. 22 | Cash, refund to association account, dues Mass. Agr. Coll. | 5.00 | 288 |
| Mar. 22 | Industrial Printing Co., bill of 11-16-29..... | 1,073.86 | 289 |
| Mar. 26 | Marie-Alice Bates, office expenses..... | 50.00 | 290 |
| Apr. 2 | Industrial Printing Co., bill of 12-11-28..... | 38.25 | 291 |
| Apr. 25 | Cash, refund to association account, dues Ga. Dept. of Agr. | 5.00 | 292 |
| May 2 | Ace Letter Service, cards and errata sheets..... | 17.50 | 293 |
| May 2 | Williams and Wilkins Co., "Physics and Biochemistry of Bacteria"..... | 5.62 | 294 |
| May 2 | Industrial Printing Co., bills, 4-16-29 and 10-25-28..... | 83.00 | 295 |
| May 15 | N. Dakota Agr. College, back numbers of <i>Journal</i> | 4.00 | 296 |
| May 15 | Industrial Printing Co., bill 4-16-29..... | 1,086.55 | 297 |
| May 22 | Industrial Printing Co., bill 5-15-29..... | 19.27 | 298 |
| May 22 | Marie-Alice Bates, office expenses..... | 50.00 | 299 |
| June 4 | J. Baumgarten & Sons Co., rubber stamps..... | 2.25 | 300 |
| June 26 | Postmaster, Washington, D. C., box rent, quarter ending 5-30-29..... | 2.00 | 301 |
| June 26 | Industrial Printing Co., bill of 6-18-29..... | 1,073.44 | 302 |
| July 1 | State of Ga. rebate on dues, duplicate payment..... | 5.00 | 303 |
| July 29 | Industrial Printing Co., fire insurance, storing <i>Methods</i> | 3.00 | 304 |
| July 29 | Industrial Printing Co., bill of 7-20-29..... | 60.70 | 305 |
| July 29 | Marie-Alice Bates, office expenses..... | 50.00 | 306 |
| Sept. 10 | Industrial Printing Co., bill of 9-6-29..... | 40.20 | 307 |
| Sept. 10 | Industrial Printing Co., bill of 8-22-29..... | 734.84 | 308 |
| Sept. 25 | Postmaster, Washington, D. C., box rent, quarter ending 9-30-29..... | 2.00 | 309 |
| Sept. 25 | Turner Sub. Agency, N. Y. C., refund for cancelled subscription..... | 4.00 | 310 |
| Sept. 27 | Wm. B. Kohn, refund for <i>Methods</i> returned..... | 5.00 | 311 |
| Oct. 1 | Ace Letter Service, billheads..... | 16.00 | 312 |
| Oct. 14 | Marie-Alice Bates, office expenses..... | 50.00 | 313 |
| | Plus bank balance, October 15, 1929..... | 1,746.56 | |
| Total | | \$7,855.34 | |

such from May, 1928, that I feel it unwise to make the Washington trip. Will you put this matter before the proper officials that they may appoint another chairman and tell them to feel perfectly free to drop me from the Committee if they so desire.

C. H. JONES.

Action has been taken on this matter and Dr. Haskins has been appointed chairman of this committee in place of Dr. Jones. I hope that before we adjourn the Resolutions Committee will bring in a resolution extending to Dr. Jones the sympathy of the association.

A matter that was brought before the Executive Committee, which is of general interest to the association, is the desire of the Board of Editors of *The Journal* to extend the usefulness and circulation of that publication. It was suggested, and the suggestion met with the approval of the Executive Committee, that there should appear in *The Journal* editorials by members of the board, or by others, on timely topics of interest to the personnel of this organization. It was also the sense of the Executive Committee, and the plan was referred to the Board of Editors for consideration, that if possible the subject matter be arranged so that the proceedings can be published in the first or perhaps first and second issues of *The Journal*. The third and fourth issues would then be devoted to contributed papers. There is a feeling that the proceedings are not available promptly enough after the meeting. With a quarterly journal you will understand that it is rather difficult to meet all these demands. The Chairman of the Board of Editors, Mr. Deemer, will make his report in the usual way, so I will not read his statement to the Executive Committee.

The treasurer's report is prepared in three parts. An operative account is maintained in which is reported the activities of the association proper. It is distinct from the account of *Methods of Analysis* and *The Journal*. When the revision of "Principles and Practice of Agricultural Analysis" is effected, it will be necessary to have a separate account opened for that.

It might also be well at this time to call your attention to the fact that *The Journal* is not self-supporting. Were it not for the income from the sale of *Methods of Analysis*, *The Journal* could not be printed. I should like to ask every member to help increase the circulation. I shall not read all the items of the financial statement because there is quite a long list of vouchers. These are all in due form and will be presented to the Auditing Committee.

I wish to call your attention to the fact that we are operating a business of between \$8,000 and \$10,000 annually, which is the main argument for a proper coordination of our activities according to the plan which has been approved by the Executive Committee.

By order of the Executive Committee, at the meeting in 1926, after the matter had been brought to its attention and thoroughly discussed,

the Treasurer was authorized to open a savings account with the Montgomery Building and Loan Association. That fund now amounts to \$1,484.34. We still have 890 copies of *Methods of Analysis*, 1925 edition, which will, we expect, just about supply the demand for the next twelve or fourteen months until the new edition is available. There is also on hand about 13,000 copies of various issues of *The Journal*. The Treasurer's account, which follows, contains a statement of assets and liabilities in usual form and includes the approximate value of the inventory.

STATEMENT OF OPERATING ACCOUNT.

OCTOBER 1, 1928, TO OCTOBER 15, 1929.

RECEIPTS—ASSOCIATION.

| | | | |
|--------|--|----------|----------|
| 1928 | | | |
| Oct. 1 | Bank balance | \$421.75 | |
| | 1928 dues from institutional members, 62 at \$5.00 | 310.00 | |
| | Reprint, O. Schreiner | 2.00 | |
| | Allowance on bill, Industrial Printing Co. | 5.75 | |
| | | | \$739.50 |

DISBURSEMENTS.

| | | | |
|----------|--|----------|--------------|
| 1928 | | | Check No. |
| Oct. 23 | Marian E. Lapp, expenses, 1928 meeting | \$30.00 | 69 |
| Nov. 5 | Estelle M. McCoy, services previous to and at 1928 meeting | 73.13 | 71 |
| Nov. 5 | Marian E. Lapp, expenses, 1928 meeting | 12.10 | 72 |
| Dec. 15 | The Montgomery Mutual Bldg. and Loan Assn. | 300.00 | 73 |
| 1929 | | | |
| Sept. 10 | Industrial Printing Co., bill 8-29-29 | 37.75 | 74 |
| Sept. 10 | Stamps for mailing programs | 15.00 | 75 |
| Sept. 10 | Refund to <i>Journal</i> account, Ga. Dept. of Agr. | 5.00 | 76 |
| Oct. 1 | Ace Letter Service, registration cards | 5.75 | 77 |
| | | \$478.73 | |
| Oct. 15 | Bank balance | 260.77 | |
| | Total | \$739.50 | |

SUMMARIZED STATEMENT.

ASSETS.

| | |
|--|------------------|
| Operating account balance | \$260.77 |
| Publications account balance | 1,746.56 |
| Savings account (Montgomery Bldg. and Loan Assn.) | 1,434.84 |
| Inventory, | |
| 890 copies <i>Methods</i> , approximately | \$2,002.50 |
| 13,623 (approximately) copies back numbers of <i>Journal</i> | 10,217.25 |
| | <u>12,219.75</u> |
| | \$15,661.92 |

LIABILITIES.

| | |
|--------------------------------|--------------------|
| Bills payable: | |
| Vol. XII, No. 4 | \$955.34 |
| Badges, 1928 meeting | 30.32 |
| Total | <u>\$985.66</u> |
| Total assets | \$15,661.92 |
| Total liabilities | 985.66 |
| Balance | <u>\$14,676.26</u> |

REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS.

Once more the committee was called upon to record the passing of one of its associates. On the eve of assembling for the session just closed, there occurred the death of R. Wilfred Balcom, valued and beloved member of the committee and for many years an active worker in this association. Fitting resolutions expressive of the loss sustained through Dr. Balcom's death were adopted.

Since the 1928 convention of this association two meetings of the committee have been held, one during the week of April 8, the other in the week of October 21.

APRIL MEETING.

At the April meeting conferences were held in connection with requests on the part of the beverage industry for the formulation of definitions and standards for these products. Groups of manufacturers were heard representing two types of beverages: (1) drinks based upon fruit juices, with water, and with or without added sugar and/or carbonation, and (2) the more largely sold type popularly known as "soda water" or "pop", consisting of flavored and sweetened carbonated water. A proposed definition for grape juice was formulated and adopted for further consideration.

Similar action was taken at this meeting for the cereal products variously known as "whole wheat flour", "entire wheat flour", and "graham flour", or "graham"; also there was formulated a proposed revision of the present definition and standard for common wheat flour, or white flour. As is well known, much confusion has long existed in regard to the first-named flours. Strictly speaking, the terms "whole wheat", "entire wheat" and "graham" mean the same thing, and should be referable to the entire product resulting from the grinding of the whole wheat berry after suitable cleaning by a process known as "scouring". In practice it has been the custom for millers to remove varying proportions of the bran coat, and in the case of graham much of the flour sold under this name to the baking industry has consisted of so-called "shovel graham", i. e., the product resulting from the blending of one or more types of white flour with bran.

Fortunately at the present time, owing to a growing disposition on the part of members of the milling industry to recognize the need of reform, the problem of a definition and its enforcement is somewhat less difficult than formerly, and there is promise of early progress to that end, although the path is still beset with difficulties. Pending this adjustment as to the flours, obviously the final correction of the corresponding situation which exists as regards the names of breads baked

from them must wait. However, at this meeting considerable preliminary consideration was given to this item.

The subject of a definition and standard for ice cream once more engaged the attention of the committee. No satisfactory solution of the peculiar difficulties which attach to this proposal seemed feasible, notwithstanding the extensive study that has been given it, and accordingly the committee unanimously voted its indefinite postponement.

Proposals were received for consideration of definitions for shortening, for sweet cream butter, and for sauerkraut juice. The matter of ash limits for mace and for cloves was brought up and was again tabled pending further study.

OCTOBER MEETING.

The subject of ice cream came up again. The committee agreed that in view of the existing situation with respect to this product, and particularly in view of the current legislation in the various States, no good purpose would be longer served by the retention of the definition and standard adopted many years ago, and accordingly its deletion was voted. Reaffirmation of the action taken at the April meeting, respecting the proposed definition and standard, was also made.

The definition and standard for mayonnaise, adopted last year, was given consideration, and this item was slightly amended, the wording being so modified as to exclude the possible misuse in this product of varieties of spices, the only purpose of which could be to impart a yellow coloration in simulation of egg.

Further consideration was given to the subject of wheat flours, and proposed definitions for reference to the industry were formulated for (1) whole wheat flour, entire wheat flour, unbolted graham flour, graham flour; (2) for bolted graham flour; and (3) for flour, wheat flour, or white flour.

The committee being agreed that no substantial demand exists at this time for a definition and standard for sauerkraut juice, this item was tabled.

A revision was made of the definition for coffee to include the variety known as robusta, which is a legitimate member of the coffee family and is now being extensively imported. The following definition was adopted:

Coffee is the seed of cultivated varieties of *Coffea arabica*, *C. liberica*, and *C. robusta*.

(a) *Green coffee, raw coffee, unroasted coffee*, is coffee freed from all but a small portion of its spermoderm and conforms in variety and in place of production to the name it bears.

(b) *Roasted coffee, "coffee"*, is properly cleaned green coffee which by the action of heat (roasting) has become brown and has developed its characteristic aroma.

The definition of skimmed milk (B. a-4)¹ was amended to harmonize with the form now occurring in most state laws, as follows:

Skim-milk, skimmed milk, is that portion of milk which remains after removal of the cream in whole or in part.

The definition of pasteurized milk (2)¹ was also revised to bring it in conformity with the present authoritative stipulation for this process, as follows:

Pasteurized milk is milk every particle of which has been subjected to a temperature not lower than 142°F. for not less than 30 minutes, and then promptly cooled to 50°F. or lower.

Without essential alteration in principle, the wordings of the definitions of milk (1) and of milks other than cow's (7) were changed slightly in the interests of clarity and of improved construction.

Affirmative action on a proposal to include lactates as an acidulant for baking powders was deferred, as was the matter of a definition for sweet cream butter. Following further consideration the proposed definition for grape juice, as formulated during the April meeting, was reaffirmed as a basis for future action. A tentative definition for orange juice and a proposed generic definition for fruit juice were prepared for consideration by the industry. It is proposed that these shall serve as the foundation for a series of beverage definitions for which there has come to be a substantial need on the part of officials as well as of the industry.

C. D. HOWARD.

E. M. BAILEY.

G. G. FRARY.

Approved.

No report was given by the Chairman of the Committee on Sampling.

No report was given by the Chairman of the Committee on Bibliography.

W. W. SKINNER: Several questions which have been asked since the report of the Executive Committee was presented leads me to believe that an additional statement concerning the volumes of "Principles and

¹ S. R. A., F. D. No. 2, 1927, p. 5.

Practice of Agricultural Analysis", which are now being revised, is in order. All the material for Volume II has been received, and a large part of it is edited. It is expected that this material will be delivered to the publishers within a few weeks. The rewriting of these volumes has been a much larger task than was anticipated. Volume II has been rewritten under the direction of the Board of Editors by the following collaborators:

| | |
|-----------------|-----------------|
| R. N. Brackett | J. S. McHargue |
| H. B. McDonnell | A. M. Peter. |
| R. Merz | C. C. McDonnell |
| W. H. Ross | J. J. G. Graham |
| G. S. Fraps | C. M. Smith |
| P. E. Howard | E. L. Griffin |
| C. W. Whittaker | B. B. Ross |

We are pleased with the splendid cooperation which we have received from these collaborators. We shall be able to take a great deal of pride in the production of this volume, and I believe that the members of the association will find it a book of reference which no member in this phase of work can afford to be without. The part on insecticides is entirely new and its like does not exist anywhere; it should be exceedingly valuable. I am saying this deliberately because I am taking this opportunity to do a little advertising. I believe that this is going to be a very worth-while effort on the part of the members of the association.

In regard to Volume III, the whole book is being rewritten. It is set up in thirty-eight chapters, twenty-one of which had been received before this meeting, one is just now handed in, and two other contributors have their parts finished. I have sent out a call to have all the copy in by December 1, and it is hoped to have this material in the publishers' hands by March 1st. There is some question concerning the size of Volume III. To keep the cost down so as to sell the book for \$5.00, the publishers insist that the pages must be kept under 700, or with cuts 750 pages. It is now believed that we cannot get this material properly presented under 1300 or 1400 pages. We are therefore confronted with the necessity of cutting the data very materially, or publishing Volume III in two parts. This is a matter to which we are giving a great deal of thought. This arrangement will make a set of four books of \$5.00 each, a cost of \$20, and the publishers think this is rather high.

I believe that Volume III will be an unusually valuable and worth-while production, a book sponsored by this association which will have as authors members of the association recognized as leaders in the fields to which the subject matter pertains.

REPORT OF AUDITING COMMITTEE.

The Auditing Committee has examined the accounts of *The Journal* and *Methods of Analysis*, covering the period from October 1, 1928, to October 15, 1929, and found the same to be correct as reported.

The committee has also examined the accounts of W. W. Skinner, Secretary-Treasurer, covering the period from October 1, 1928, to October 15, 1929, and found the same to be entirely correct.

A. E. PAUL.

L. E. BOPST.

Approved.

REPORT OF COMMITTEE ON NECROLOGY.

During the past twelvemonth death has removed several distinguished members of our association.

The important part played by J. K. Haywood and Anthony McGill in the collection of accurate data, in the development of trustworthy methods of analysis, and, it may be said, in enhancing the dignity of their chosen profession, has been commemorated in biographical notices published in *The Journal* during the year. The committee, feeling that this work of appreciation has been in the hands, not only of friends, but of competent critics, will do no more now than place its modest wreath where more worthy tributes already lie.

Just as the members of this association were preparing to leave their homes for this meeting in Washington, came news that R. W. Balcom of the Food, Drug and Insecticide Administration, U. S. Department of Agriculture, and Edwin Le Fevre of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, had suddenly been called to lay down their tools, for the day of their labor had come to its end. There has been no time for your committee to prepare adequate reports upon the work of these men or upon their personal characteristics.

Dr. Balcom was particularly active in the work of this association for many years, serving as referee and as Chairman of the Board of Editors of *The Journal*. Whatever the duty assigned, it was performed with zeal and meticulous care. He was dignified and reticent, even shy, perhaps a characteristic of workers in science, who are not actuated by maudlin sentiment. His personal friendships were matters of slow development, and he might well be proud who was numbered among those to whom an entirely personal friendliness was shown.

Dr. Le Fevre was a bacteriologist and therefore his work was not closely connected with the activities of the association. However, he often attended the meetings and was held in high esteem by his fellow scientists.

Let us, then, show reverence for our calling and do honor to ourselves by acknowledging the high merit of our comrades of yesterday!

Approved.

W. W. RANDALL.
H. C. LYTHGOE.

REPORT OF NOMINATING COMMITTEE.

The Nominating Committee desires to place in nomination the following names:

President: E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

Vice-President: H. D. Haskins, Agricultural Experiment Station, Amherst, Mass.

Secretary-Treasurer: W. W. Skinner, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee:

F. C. Blanck, Washington, D. C.

J. W. Kellogg, Harrisburg, Pa.

A. E. Paul, Chicago, Ill.

Ex-Officio Member of Executive Committee:

H. B. McDonnell, College Park, Md.

C. D. HOWARD.
W. H. MACINTIRE.
H. A. LEPPER.

It was moved, seconded and carried that the secretary be directed to cast a unanimous ballot for the officers nominated.

H. B. McDonnell: It is wonderful what unanimity of action can occur in a group of this kind. It takes the thorough work of the referees and their assistants and of the various committees to make things work smoothly, and it gives me great pleasure that the work has been handled so well. Dr. Bailey, I hope you will have the whole-hearted and unanimous support in the work that I have received, and that the work will be as pleasant for you as it has been for me.

E. M. Bailey: I am sure that you and the committee have been generous far beyond what I deserve, so far as contributions to the association are concerned. However, due to the fact that the New England conscience may slip a little sometimes, I shall be justified in accepting this honor at your hands, whether I deserve it or not. If I thought that by making a speech now, the Nominating Committee would let me out next year, I should be tempted to do so. I should much rather talk to this small group. However, great as this honor is and deeply as I appreciate it, I do not propose to make two speeches to pay for it. So, just expressing my profound appreciation for this courtesy and honor that you have given me, I thank you very much.

REPORT OF COMMITTEE ON RESOLUTIONS.

(1) *Resolved:* That to the Hon. R. W. Dunlap, Assistant Secretary of Agriculture, we express our thanks for his willingness to take from a busy life the time required to extend to us his hearty greeting. Realizing the close relation which necessarily exists between the Department and this Association, we express the hope that such greetings may continue to encourage us as the years pass.

(2) *Resolved:* That to our President, Dr. H. B. McDonnell, we express our appreciation of the painstaking research his interesting address has described. The close association existing among the data which the fields of chemistry, physics and biology provide—their interpenetration, indeed—adds a new wonder in our study of problems directly pertaining to life and its better maintenance.

(3) For the first time in more than forty years this association has, on assembling, been confronted with the disappointing announcement: "Dr. Wiley cannot be present". Nor is this all. The message now must be: "Dr. Wiley is too ill to leave his bed or even to speak to life-long friends". Does this mean that a tradition must now be surrendered, that an epoch (in a sense) has come to its end? Therefore, be it

Resolved: That this association extends to its Honorary President its affectionate sympathy during this trying period of his illness, and expresses the earnest hope that he will appear before us a year hence with all his old enthusiasm and vigor unimpaired.

(4) Throughout this session the association has, to its great disadvantage, been deprived of the counsel of one of its most honored members. Therefore, be it

Resolved: That we convey to Dr. C. H. Jones an expression of our regret that illness has prevented his coming to Washington at this time, and of our hope that he will experience a speedy and complete recovery.

(5) *Resolved:* That to Dr. Skinner we express our thanks for all the detailed work he has performed without cessation as Secretary-Treasurer of this association. Presidents may come and presidents may go, but such secretaries must not be allowed to retire!

(6) *Resolved:* That to Miss Lapp we extend our sincere thanks for her skillful planning in preparation for our meeting, as well as for her careful editorial work upon *The Journal*. Few members realize how invaluable her labors have been in promoting the dignity of our association.

(7) *Resolved:* That to the Management of the Raleigh Hotel we again express our thanks for the use of rooms and appurtenances so necessary for a successful convention.

Approved.

W. W. RANDALL.
H. C. LYTHGOE.

CONTRIBUTED PAPERS.

SOME OBSERVATIONS ON THE DETERMINATION OF LEVULOSE WITH CUPRO-POTASSIUM CARBONATE SOLUTION*.

By H. A. SCHUETTE and JENNETTE N. TERRILL (Laboratory of Foods and Sanitation, University of Wisconsin, Madison, Wis.).

Fifty-three years ago Soldaini (1)† introduced into sugar chemistry the so-called cupro-carbonate solution as an improvement on the oxidizing agents of this type then extant. Although divergent opinions were subsequently expressed as to the merits of this new reagent (5-11) and several modifications centering around its composition were made‡, the view persisted that his procedure for determining reducing sugars avoided certain errors resulting from the use of Fehling's solution for similar purposes. Soldaini's solution appealed to chemists because it was deemed superior to Fehling's solution from the standpoint of (1) stability, (2) its apparent passivity towards sucrose, and (3) its extreme sensitiveness to reduction by invert sugar. Inasmuch as Ost (13a) revised the formula for making this copper solution, as modified in turn by those who followed Soldaini in this field (4, 5, 10-12), the later literature invariably makes reference to this reagent under his name. Unlike others of its type, it is characterized by the absence of hydroxylated organic compounds.

The equilibrium conditions within this reagent were apparently not understood at the time of its introduction and, it seems, little progress has been made in this direction since then. That there is an obvious need for such information is apparent in the light of certain observations noted in this paper.

Interest has centered anew around this reagent with the announcement by Nyns (23) that under closely guarded conditions as to time, temperature, and concentration it shows a selective reactivity towards levulose to the exclusion of dextrose and certain other aldose sugars, as well as lactose and sucrose. Since a practical application of this procedure suggested itself in the case of honey, a critical study of Nyns' method was undertaken in the hope that a solution of the problem of determining a ketose in the presence of an aldose and a disaccharide had been found.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1929.

† Numbers refer to the bibliography on the Soldaini-Ost Solution at the end of this article.

‡ A review of the literature on this solution reveals the fact that its copper content has been made to vary between 0.785 and 12.5 mg. per cc.

The observations of the writers, which substantiate those of Jackson (24), do not parallel the conclusions reached by Nyns. This, however, is deemed to be of secondary importance to the discovery of the fact that there apparently exists an unsuspected source of error in the use of copper-potassium carbonate solutions for the determination of reducing sugars, particularly levulose.

STABILITY OF THE REAGENT.

It is frequently stated in the literature (4, 9, 10) that the reagent of Soldaini, as variously modified before Ost (13, 20) improved it, is stable. Ost himself advanced the same claim, a view which Schmoeger (17) held untenable. That the two solutions used by Nyns (23) do not possess the stability claimed for them has been indicated by Jackson (24).

Stability tests were carried out in this laboratory by observing over a 20-month period the behavior of six of the seven solutions whose compositions are summarized in Table 1. They were prepared exactly as described by their sponsors, and for the first six months were stored in the dark. It is significant to note that all the authors recorded in the table kept constant the concentration of alkaline carbonate, viz., 100 and 250 grams of potassium bicarbonate and carbonate, respectively, and that only the copper sulfate content was varied. In the light of these data it appears that a copper sulfate content of 15.7 grams per liter is the limiting concentration beyond which solutions of this type cannot be expected to show a reasonable degree of stability.

TABLE 1.
Stability of copper-potassium carbonate solutions.

| AUTHOR | SOLUTION | $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ GRAMS PER LITER | OBSERVATION | REFERENCE TO BIBLIOGRAPHY |
|--------------|----------|--|--------------------|------------------------------|
| Ost | | 23.5 | precipitate formed | 13a, b |
| Ost | I | 17.5 | precipitate formed | 20 |
| Ost | II | 3.6 | stable | 20 |
| Beyersdorfer | I | 15.71 | stable | 22 |
| Beyersdorfer | II | 3.14 | stable | 22 |
| Nyns | I | 25.3 | precipitate formed | 23 |
| Nyns | II | 15.0 | | 23 |

REAGENTS.

Inasmuch as Nyns' modifications of the Ost solutions had been found to lack a satisfactory degree of stability, they were made up in two parts. One solution contained that quantity of copper sulfate and the other that of potassium carbonate and bicarbonate necessary to produce, when mixed in the proper proportions, a reagent of the same composi-

tion as used by its originator. These ends were met by mixing, just before use, four volumes of the alkaline carbonate solution (312.5 grams of K_2CO_3 and 125 grams of $KHCO_3$ per liter) with one volume of the copper solution (126.5 grams or 75 grams of $CuSO_4 \cdot 5H_2O$ per liter, as the case might be).

EXPERIMENTAL PROCEDURES.

Exactly 20 cc. of the levulose solution (Bureau of Standards quality), containing on the one hand not over 100 mg. of this sugar for reduction of the stronger copper sulfate solution (solution I) or, on the other hand, 60 mg. for reduction of the weaker one (solution II), was added to 50 cc. of the freshly mixed Ost solution, which had been previously brought to the desired temperature by immersion in a water-bath thermostatically controlled at $48.9^\circ C. \pm 0.1$. The reaction flasks, lightly corked and submerged to within one inch of the top, were kept in the bath for 2.5 hours, after which the precipitated cuprous oxide was determined gravimetrically.

It was observed in the first case that reduction of copper solution I was complete with a levulose concentration lying between 90 and 100 mg. Similarly 55 mg. was found to be approximately the maximum quantity of this sugar which the less concentrated copper solution was capable of oxidizing.

Data obtained from the reduction of both copper solutions by the writers appeared to represent accurately the relationships obtaining between levulose and the several Ost solutions. There was no reason to take a different view since all determinations had been made in quadruplicate with excellent agreement in cuprous oxide recovered.

A chance observation made during the course of this investigation brought to light a factor that apparently has been overlooked by other investigators. The filtrate from one of the reductions was set aside till morning, when it was observed that more cuprous oxide had settled out. That this cuprous oxide was not due to a photochemical decomposition of the reagent was indicated when controls, left standing on the laboratory table fully exposed to the light, showed the same reaction as those put aside in the dark. A typical set of data that illustrate the effect of a delayed filtration is reproduced in Table 2.

Some of the cuprous oxide obviously remains in colloidal dispersion until equilibrium is attained in the reaction mixture, an observation based upon the Tyndall phenomenon. That it is unique with this type of reagent was verified when similar reductions of the three modifications of Soldaini's solution introduced by Ost (13a, 15) and that form of Ost's solution introduced by Beyersdorfer (22) and containing 15.7 grams of copper sulfate, all showed the same phenomenon, and when no such condition was detectable in the filtrates resulting from the reduction of

TABLE 2.

Comparison of the observed levulose-copper equivalents of Ost's solutions obtained under different conditions of filtration.

| LEVULOSE mg. | SOLUTION I | | SOLUTION II | |
|-----------------|-------------------------|-----------------------|-------------------------|-----------------------|
| | Immediate filtration | Delayed filtration | Immediate filtration | Delayed filtration |
| | mg. Cu | | mg. Cu | |
| 10 | 28.69 | 29.57 | 31.26 | 31.26 |
| 20 | 60.25 | 62.20 | 69.63 | 69.63 |
| 30 | 96.20 | 97.16 | 105.12 | 105.12 |
| 40 | 131.62 | 139.05 | 137.70 | 138.24 |
| 50 | 171.43 | 179.31 | 167.00 | 167.51 |
| 55 | not determined | | 180.63 | 180.63 |
| 60 | 206.65 | 217.96 | | |
| 70 | 255.65 | 258.86 | | |
| 80 | 279.56 | 293.10 | | |
| 90 | 315.30 | 316.20 | | |

Fehling's solution by the procedure of Munson and Walker*. Washing the precipitated cuprous oxide with either the carbonate or bicarbonate of potassium (15 per cent solution) failed to prevent the appearance of the colloidal form. Whatever merit obtains in this practice may be generalized with the statement that equilibrium appears to be reached sooner below a copper sulfate level of 17.5 grams than above it. The minimum time in which this condition is reached is apparently 24 hours; for greater concentrations it is at least 48 hours.

From each series of results recorded in Table 3, columns two and five, respectively, there was deduced a formula for calculating the equivalent amount of levulose from any given weight of copper within the limits of the method.

TABLE 3.

Levulose-copper equivalents of Ost's solutions when filtration was delayed for 48 hours.

| LEVULOSE mg. | SOLUTION I | | | SOLUTION II | | |
|-----------------|--------------------------|---------------------------|---------------------------------------|--------------------------|---------------------------|---------------------------------------|
| | Cu _{obs} mg. | Cu _{calc} mg. | Cu _{obs} -Cu _{calc} | Cu _{obs} mg. | Cu _{calc} mg. | Cu _{obs} -Cu _{calc} |
| 10 | | | | 31.26 | 29.21 | -2.05 |
| 20 | 62.20 | 62.20 | 0.00 | 69.36 | 69.16 | -0.20 |
| 30 | 97.16 | 100.68 | +3.52 | 105.12 | 105.50 | +0.38 |
| 40 | 139.05 | 139.16 | +0.11 | 138.24 | 138.25 | +0.01 |
| 50 | 179.31 | 177.64 | -1.67 | 167.51 | 167.41 | -0.10 |
| 55 | not determined | | | 180.63 | 180.63 | 0.00 |
| 60 | 217.96 | 216.12 | -1.84 | | | |
| 70 | 258.86 | 254.60 | -4.26 | mean deviation | | |
| 80 | 293.10 | 293.10 | 0.00 | | | ±0.45 |
| | mean deviation | | | | | ±1.63 |

If levulose is represented by x and the corresponding amount of copper by y , the relation between them for solution I may be expressed by the formula of a straight line, $y = mx + b$. Inasmuch as a graph of the observed levulose-copper equivalents of solution II (Table 3, columns

* *J. Am. Chem. Soc.*, 28, 663, (1906).

one and five) resolves itself into a parabola, the equation $y = a + bx + cx^2$ is pertinent. The equation for the stronger copper solution resolves itself into the expression $y = 3.6555x + 12.86$. For the weaker solution it is $y = -14.375 + 4.535x - 0.018x^2$. By substituting these values in the foregoing equations, the calculated weights of copper recorded in columns 3 and 6 were obtained. This method of procedure distributes the experimental errors over the whole range.

The mean deviation of equivalent copper weights as found for these reagents is ± 1.63 mg. for the stronger solution and ± 0.45 mg. for the weaker. It follows, therefore, that as between a choice of the two for analytical work, preference might well be given that solution containing 15 grams of copper sulfate. Then, too, the fact that equilibrium is reached in the reaction mixture approximately twice as fast with the weaker as with the stronger solution suggests another recommendation. In the light of the observations made of the behavior of these reagents, however, it would seem that no very useful ends are gained by recourse to procedures characterized by the retarded effects which these have shown. The difficulty might be obviated if there could be developed a volumetric procedure for the determination of the precipitated cuprous oxide without previous filtration, as for example after the manner of Schaffer and Hartman*.

SUMMARY.

In an attempt at an experimental verification of the statement (23) that a modified Soldaini-Ost cupro-carbonate solution can be made to show a selective reactivity towards levulose in the presence of dextrose, the observation was made that there exists an apparently unsuspected source of error in the use of certain sugar-oxidizing reagents of this type. This was found to be true when these reagents were used for the gravimetric determination of levulose. The error in question is due to the formation, in part, of colloidal cuprous oxide, which remains dispersed in the filtrate for approximately 24-48 hours, depending upon the concentration of the copper sulfate in the reagent that was used.

The levulose-copper equivalents of two modifications (23) of the Soldaini-Ost solution have been determined. These equivalents are only valid if reduction is carried out for 2.5 hours at a temperature of 48.9° and if filtration is delayed sufficiently long enough to allow for the complete flocculation of the reduced copper oxide. Mathematical expressions have been derived for these equivalents.

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DETERMINATION OF CITRIC ACID IN FRUITS AND FRUIT PRODUCTS.

By B. G. HARTMANN and F. HILLIG (Food Control Laboratory, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C.).

In a previous paper¹ the authors reported the results of an investigation of the Stahre Reaction in which it was found that under controlled conditions this reaction accurately determines citric acid. Therefore this information was used as the basis in formulating a method for the determination of citric acid in fruits and fruit products². In the procedure an adjustment of solids and citric acid content in the portion of the material taken for analysis is prescribed. Although the method has proved satisfactory, further investigation showed that equally accurate results may be obtained by applying the pentabromacetone procedure to the isolated acids precipitable by lead acetate and thereby avoiding any adjustment. It is apparent that such a procedure is preferable to the one published, since the specificity of the reaction is not sacrificed, and the method is made qualitative as well as quantitative.

The precipitation of citric acid as pentabromacetone in the procedure described in this paper is essentially the same as that given in the first publication. The preparation of the portion for analysis involves the removal of pectin and the separation of the organic acids. It seems unnecessary to go into detail regarding the experimental work leading up to the best conditions for the isolation of the organic acids. It was found that the lead salts of the acids are practically insoluble in strong alcohol. The removal of pectin is necessary because this substance has a tendency to form colloidal solutions which filter with difficulty, particularly in the case of the decomposition of the lead salts with hydrogen sulfide. Sulfuric acid is added prior to the precipitation of pectin with alcohol in order to decompose the salts of organic acids which are normally present in fruit juices and which may, in part, be precipitated by the alcohol. Since esters of citric acid do not form pentabromacetone, it is necessary to saponify materials containing alcohol.

PROPOSED METHOD FOR THE DETERMINATION OF CITRIC ACID IN FRUITS AND FRUIT PRODUCTS.

PREPARATION OF SAMPLE.

With products containing large quantities of sugars, such as jams and jellies, use 200 cc. of a sample solution prepared as described in *Methods of Analysis*, A. O. A. C., 1925, 209. In the case of fruit juices or products of similar nature, use a portion of the material containing not more than 200 mg. of acid calculated as citric acid.

Determine the acidity of the portion taken in terms of normal acid (titratable acidity). Adjust to a volume of approximately 35 cc. either by evaporation or by the addition of

¹ *This Journal*, 10, 284 (1927).

² *Ibid.*, 11, 257 (1928).

water, add 3 cc. of normal sulfuric acid, and heat to 50°C. Designate the acidity of the solution as "A" (titratable acidity plus 3). Pour the contents of the beaker into a 250 cc. volumetric flask. Rinse the beaker with about 15 cc. of warm water and finally with 95 per cent alcohol, make to mark with 95 per cent alcohol, shake, allow to stand 15 minutes, and filter through absorbent cotton. Prepare the filter by shaping the cotton into a large-sized funnel, using as thin a layer of cotton as is practicable. (Filtration is usually fast, but occasionally it is necessary to renew the cotton. In the latter case the liquid is squeezed onto the new filter.) During filtration cover the funnel with a watch-glass to retard evaporation of alcohol. Transfer 200 cc. of the clear filtrate to a 16 ounce centrifuge bottle.

In the case of products containing alcohol, saponification is necessary. For this purpose adjust the volume of the portion taken to about 35 cc., add 3 cc. of normal KOH alkali in excess of that required for neutralization, heat to boiling, and allow to stand overnight. Add normal sulfuric acid equal to the total quantity of normal alkali added and 3 cc. in excess. Transfer to a 250 cc. volumetric flask as described previously. "A" in this case equals the number of cubic centimeters of normal sulfuric acid added plus the titratable acidity of the material.

REAGENTS.

Potassium bromide solution.—Dissolve 15 grams of potassium bromide in 40 cc. of water.

Potassium permanganate solution.—Dissolve 5 grams of potassium permanganate in water and dilute to 100 cc.

Ferrous sulfate solution.—Dissolve 40 grams of ferrous sulfate in 100 cc. of water containing 1 cc. of concentrated sulfuric acid.

Lead acetate solution.—Dissolve 70 grams of lead acetate in water, add 1 cc. glacial acetic acid, and dilute to 250 cc.

DETERMINATION.

To the material in the centrifuge bottle, add a quantity of the lead acetate solution equal to 0.8A, shake vigorously for 2 minutes, and centrifuge at about 900 r. p. m. for 15 minutes. Carefully decant the supernatant liquid from the precipitated lead salts. If sediment lifts, repeat the centrifuging, increasing the speed and time. Allow to drain thoroughly by inverting the bottle for several minutes. To the material in the centrifuge bottle add 150 cc. of 80 per cent alcohol, shake vigorously and again centrifuge, decant and drain. Transfer the salts to a 400 cc. beaker with about 150 cc. of water. Warm, and pass in a rapid stream of hydrogen sulfide until the solution is cool, stirring frequently. Transfer to a 250 cc. volumetric flask, make to mark with water, and filter through a folded filter. Transfer 225 cc. of the filtrate to a 500 cc. Erlenmeyer flask, add several small glass beads to facilitate boiling, and evaporate to about 75 cc. Cool, and add 10 cc. of dilute sulfuric acid (1 + 1) and 5 cc. of the potassium bromide solution. Heat the mixture to 48°–50°C. and maintain this temperature for 5 minutes. To the warm solution add immediately 50 cc. of the potassium permanganate solution; shake vigorously in the stoppered flask for about 1 minute, releasing the pressure frequently; and allow to stand 4 minutes. Do not permit the temperature to exceed 55°C. (During this time there should be a heavy deposit of manganese dioxide; if necessary, add more potassium permanganate to assure an excess of the oxidizing agent. If at any time during the oxidation the precipitated manganese dioxide disappears, discard the determination and repeat, using more potassium permanganate.) Remove manganese dioxide with the ferrous sulfate solution (20 cc. is generally sufficient), cool, shake vigorously, and place in the refrigerator overnight. Filter by decantation onto a thin, tightly-tamped pad of asbestos in a Gooch crucible (it is important that filtration

be completed as quickly as possible). Note volume of filtrate and use this filtrate to transfer the precipitate to the crucible. Wash the contents of the crucible at once with 50 cc. of ice-cold water. Dry in a sulfuric acid vacuum desiccator and weigh. To remove the pentabromacetone, treat the contents of the crucible with three portions of 20 cc. each of alcohol and three portions of 20 cc. each of ether. Again dry and weigh. The difference in the two weights represents the weight of pentabromacetone. Calculate the citric acid by the following formula:

$X = 1.05 (0.424P + 0.017S)$, in which

X = milligrams of citric acid in aliquot,

P = weight of pentabromacetone in milligrams, and

S = volume of filtrate (cc.).

The pentabromacetone may be dried by aspirating with air¹. The tabulated results were obtained by using this method of drying.

The method given was tried on citric acid in pure solution, but the final correction of 1.05 was not applied. The quantity of potassium permanganate used in this case was 15 cc., which was found to be sufficient. The results are given in Table 1.

TABLE 1.

Citric acid in pure solution.(Solubility factor 1.7 mg. per 100 cc. filtrate used².)

| CITRIC ACID PRESENT mg. | CITRIC ACID DETERMINED mg. | RECOVERY per cent |
|-------------------------------|----------------------------------|----------------------|
| 4 0 | 3 0 3 0 | 75 0* |
| 20 9 | 19 2 20 0 | 93 8 |
| 51.2 | 47 4 47 8 | 93 0 |
| 98.6 | 94 2 93.9 | 95 4 |
| 153.2 | 146 8 144.7 | 95.2 |
| 196 9 | 189 7 188 0 | 95 9 |
| | Average | 94 7 |

* Not used in average.

The percentages of recovery in the various determinations, with the exception of the one containing 4 mg., agree fairly well, the average being approximately 95 per cent.

In Table 2 the data presented in Table 1 are corrected for the average loss. The results so corrected are very close to the quantity of citric acid added.

¹ *This Journal*, 10, 272 (1927).

² *Ibid.*, 271.

TABLE 2.

Results in Table 1 multiplied by 1.05 to correct for average loss of 5 per cent.

| CITRIC ACID PRESENT | CITRIC ACID CORRECTED | RECOVERY CORRECTED |
|------------------------|--------------------------|-----------------------|
| mg. | mg. | per cent |
| 4 0 | 3.2 | 80.0 |
| 20 9 | 20.6 | 98 6 |
| 51 2 | 50 0 | 97 7 |
| 98 6 | 98.8 | 100 2 |
| 153 2 | 153 1 | 100 0 |
| 196 9 | 198 3 | 100 7 |

In order to show the effect that malic and tartaric acids might have on the determination of citric acid, the data in Table 3 are presented. Apparently the presence of malic and tartaric acids does not affect the determination.

TABLE 3.

Results obtained when proposed method was used on mixtures of citric, malic and tartaric acids.

| CITRIC ACID PRESENT | MALIC ACID PRESENT | TARTARIC ACID PRESENT | CITRIC ACID DETERMINED | RECOVERY |
|------------------------|-----------------------|--------------------------|---------------------------|----------------|
| mg. | mg. | mg. | mg. | per cent |
| 4 0 | 46 7 | 47.3 | 5.3 2 7 | 4 0 |
| 98.6 | 46 7 | 47.3 | 100.0 99 9 | 100 0 |
| 196 9 | 46 7 | 47 3 | 197 0 197 0 | 101 4 100 1 |

Table 4 presents results obtained on varying quantities of citric acid added to apple jelly and blackberry jam.

TABLE 4.

Added citric acid in apple jelly and blackberry jam determined by proposed method.

| MATERIAL (30 GRAMS) | CITRIC ACID ADDED | CITRIC ACID DETERMINED | RECOVERY | CITRIC ACID IN Present | CITRIC ACID IN Material Found |
|------------------------|----------------------|---------------------------|----------|---------------------------|----------------------------------|
| | mg. | mg. | per cent | per cent | per cent |
| Apple jelly* | 7 0 | 7 3 5 9 | 6 6 | 94.3 | 0 023 |
| " | 117 1 | 113 1 113 2 | 113 2 | 96.7 | 0 377 |
| " | 228 8 | 226 5 222 1 | 224 3 | 98 0 | 0 748 |
| " | 307 8 | 296 4 297 3 | 296.9 | 96 5 | 1.026 |
| Blackberry Jam* | 7 0 | 7 3 7 5 | 7.4 | 105.7 | 0.023 |
| " | 116 5 | 114 7 114 3 | 114 5 | 98.3 | 0.388 |
| " | 233 0 | 224.0 222 1 | 223 1 | 95.8 | 0.777 |
| " | 311 0 | 307.5 303 3 | 305.4 | 98.2 | 1.037 |

* Materials yielded no precipitate of pentabromacetone.

From the data in Table 4 it is evident that the procedure is applicable to materials containing large quantities of sugar, pectin, and organic acids other than citric acid.

Finally, the determination of citric acid in four common fruit products was made. These materials were chosen because they contain organic acids commonly found in fruits and fruit products: malic, tartaric, citric and isocitric.

TABLE 5.
Citric acid in fruit products.

| MATERIAL | WEIGHT OF MATERIAL grams | ACID AS CITRIC BY TITRATION mg. | CITRIC ACID DETERMINED mg. | CITRIC ACID per cent |
|----------------------|-----------------------------|---------------------------------------|----------------------------------|-------------------------|
| Apple jelly | 30 0 | 112 6 | 0 0 | 0 0 |
| Blackberry jam . . . | 30 0 | 118 4 | 0 0 | 0 0 |
| Grape juice | 32 5 | 281 6 | 7 3 | 0 023 |
| Orange juice | 32 0 | 256 0 | 272 4 | 0 851* |

* Acid as citric by titration, 0.80 per cent

It will be noted that the percentage of citric acid determined in orange juice exceeds that indicated by titration. This is explained by the fact that orange juice contains citric acid in the combined form. The small quantity of citric acid found in Concord grape juice confirms the previous work of the authors and of E. K. Nelson regarding the presence of the acid in this juice. The apple jelly used was prepared in the laboratory from greening apples, and attention is called to the absence of citric acid in this variety. The blackberry jam was obtained on the market. According to Nelson, the acidity of blackberry is due to isocitric acid. The results show that the blackberries used to prepare the jam contained no citric acid.

SUMMARY.

A method for the determination of citric acid in fruits and fruit products has been described. The citric acid is isolated as the lead salt, and decomposed with hydrogen sulfide, and the liberated acid is determined as pentabromacetone. Malic, tartaric and isocitric acids do not interfere with the determination. The method was tried on mixtures of pure acids and on fruit products.

DETERMINATION OF TARTARIC ACID IN FRUITS AND FRUIT PRODUCTS.

By B. G. HARTMANN and F. HILLIG (Food Control Laboratory¹, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C.).

Two general procedures for the determination of tartaric acid in fruits and fruit products are available: (1) precipitation as acid potassium tartrate, and (2) precipitation as calcium racemate.

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The acid potassium tartrate procedure is official for wines¹ and has been tentatively adopted for fruits and fruit products². The determination of the acid as calcium racemate (Kling method)³ has been reported upon by Nelson, who recommended a modification for adoption as a tentative method for fruits and fruit products⁴.

Collaborative investigations have shown that the acid potassium tartrate procedure is satisfactory for wines, but not for fruits and fruit products, while the racemate procedure, as modified by Nelson, has been reported to be satisfactory for fruits and fruit products.

The unsatisfactory results which have been reported on the determination of tartaric acid in fruits and fruit products by the acid potassium tartrate procedure are in the main attributable to interference of pectin, which when precipitated tends to occlude acids other than tartaric acid, also coloring matter, thereby causing high results and making the end point in the titration inaccurate.

The removal of the interfering pectin may be accomplished by precipitation with strong alcohol. In the alcoholic filtrate the acids may then be obtained free from sugar by precipitation with lead acetate. By decomposing the lead salts with hydrogen sulfide the tartaric acid and other acids precipitable by lead acetate are secured in a fairly pure condition. The tartaric acid in the isolated acids may then be precipitated as acid potassium tartrate from strong alcohol, in which medium the salt is only sparingly soluble.

Based upon these considerations, the following method for fruits and fruit products was formulated.

PROPOSED METHOD FOR THE DETERMINATION OF TARTARIC ACID AS ACID POTASSIUM TARTRATE IN FRUITS AND FRUIT PRODUCTS.

PREPARATION OF SAMPLE.

With products containing large quantities of sugars, such as jams and jellies, use 200 cc. of a sample solution prepared as described in *Methods of Analysis, A. O. A. C.*, 1925, 209. In the case of fruit juices or products of similar nature use a portion of the material containing not more than 200 mg. of acid calculated as tartaric acid.

Determine the acidity of the portion taken in terms of normal acid (titratable acidity). Adjust to a volume of approximately 35 cc., either by evaporation or by the addition of water, add 3 cc. of normal sulfuric acid, and heat to 50°C.

Designate the acidity of the solution as "A" (titratable acidity plus 3). Pour the contents of the beaker into a 250 cc. volumetric flask. Rinse the beaker with about 15 cc. of warm water and finally with 95 per cent alcohol, make to mark with 95 per cent alcohol, shake, allow to stand 15 minutes, and filter through absorbent cotton. Prepare the filter by shaping the cotton into a large-sized funnel, using as thin a layer of cotton as is practicable. (Filtration is usually fast, but occasionally it is necessary to renew the cotton. In the latter case the liquid is squeezed onto the new filter.) During filtration cover the funnel with a watch-glass to retard evaporation of alcohol. Transfer 200 cc. of the clear filtrate to a 16 ounce centrifuge bottle.

¹ *Methods of Analysis, A. O. A. C.*, 1925, 366.

² *Ibid.*, 213.

³ *Bull. Soc. Chim.*, 1910 (4) 7: 567; 11, 886 (1912).

⁴ *This Journal*, 8: 640 (1925).

In the case of products containing alcohol, saponification is necessary. For this purpose adjust the volume of the portion taken to about 35 cc., add 3 cc. of normal KOH alkali in excess of that required for neutralization, heat to boiling, and allow to stand overnight. Add normal sulfuric acid equal to the total quantity of normal alkali added and 3 cc. in excess. Transfer to a 250 cc. volumetric flask as described previously. "A" in this case equals the number of cubic centimeters of normal sulfuric acid added plus the titratable acidity of the material.

DETERMINATION.

To the material in the centrifuge bottle add a quantity of lead acetate solution equal to 0.8A, shake vigorously for two minutes, and centrifuge at about 900 r. p. m. for 15 minutes. The lead acetate solution is prepared by dissolving 70 grams of lead acetate in water acidulated with 1 cc. of glacial acetic acid and diluting to 250 cc. with water. Carefully decant the supernatant liquid from the precipitated lead salts. If the sediment lifts, repeat the centrifuging, increasing the speed and time. Allow to drain thoroughly by inverting the bottle for several minutes. To the material in the centrifuge bottle add 150 cc. of 80 per cent alcohol, shake vigorously, and again centrifuge, decant and drain. Transfer the salts to a 400 cc. beaker with about 150 cc. of water. Warm and pass in a rapid stream of hydrogen sulfide until the solution is cool, stirring frequently. Transfer to a 250 cc. volumetric flask, make to mark with water, and filter through a folded filter. Transfer 225 cc. of the clear filtrate to a 400 cc. beaker, add several small glass beads to facilitate boiling, and evaporate to 20 cc. over a small flame. Neutralize with normal *potassium hydroxide*, using phenolphthalein as indicator, and add 3 drops of the alkali in excess. Add 2 cc. of glacial acetic acid and 80 cc. of 95 per cent alcohol slowly and with constant stirring. Chill in an ice bath, stir vigorously for 2 minutes, and place in the refrigerator overnight. Carefully decant the supernatant liquid onto a thin pad of asbestos in a Gooch crucible with a removable bottom, leaving about 25 cc. in the beaker. To the contents of the beaker add about 0.3 gram of dry purified asbestos. Mix thoroughly and transfer to the crucible with three portions of about 15 cc. each of ice-cold 80 per cent alcohol and finally wash the contents of the crucible with three portions of 15 cc. each of the ice-cold alcohol, sucking the crucible dry each time. Transfer the pad and precipitate to a 250 cc. beaker, with about 100 cc. of hot water, bring almost to boiling, and titrate with 0.1 N alkali, using phenolphthalein as indicator. To obtain the quantity of tartaric acid multiply the number of cubic centimeters of 0.1 N alkali used by 0.015.

The method was tried on solutions containing known quantities of pure tartaric acid.

TABLE 1.
Tartaric acid in pure solution.

| PRESENT mg. | DETERMINED mg. | | RECOVERED per cent |
|----------------|-------------------|-------|-----------------------|
| 9 8 | 10.1 9 4 | 9 8 | 100 0 |
| 49 5 | 47.8 47 3 | 47 6 | 96 2 |
| 148 1 | 145.1 143 5 | 144.3 | 97.4 |
| 247.3 | 239 9 241 0 | 240.5 | 97.3 |

The results indicate a small loss of tartaric acid, but whether this loss is due to incomplete precipitation or to the solubility of acid potassium tartrate in the alcoholic solution is not determinable.

In order to show the effect which malic and citric acids have on the determination, the data in Table 2 are presented.

TABLE 2.
Solutions containing tartaric, citric and malic acids.

| Tartaric mg. | ACIDS PRESENT Citric mg. | Malic mg. | TARTARIC ACID DETERMINED mg. | RECOVERED per cent |
|-----------------|--------------------------------|--------------|------------------------------------|-----------------------|
| 9 8 | 100 | 100 | 8 3 7.5 | 7.9 81.0 |
| 49.5 | 100 | 100 | 49.1 46.8 | 48.0 97.0 |
| 121.1 | 75 | 75 | 117.0 119.3 | 118.2 97.6 |
| 148.1 | 100 | 100 | 146.6 150.1 | 148.4 100.2 |
| 181.7 | 50 | 50 | 177.8 178.2 | 178 0 98.0 |
| 252 4 | 25 | 25 | 247.0 248.1 | 247.6 98.1 |

From a comparison of the results in Table 2 with those in Table 1 it is apparent that the presence of malic and citric acids in the solution does not interfere with the determination.

Table 3 presents results obtained on varying quantities of tartaric acid added to apple jelly and blackberry jam. The apple jelly was prepared in the laboratory from greening apples, and the blackberry jam was obtained in the market.

The small quantity of tartaric acid determined in the apple jelly is questioned; it is very possible that the result is incidental to the procedure, and is not due to tartaric acid. The tartaric acid determined in the blackberry jam (0.047 per cent) is believed to be substantially correct, inasmuch as duplicate determinations obtained by the Kling procedure (described in this paper) showed 0.038 and 0.036 per cent.

DETERMINATION OF TARTARIC ACID AS CALCIUM RACEMATE.

When *l*-tartaric acid is added to a solution of *d*-tartaric acid in the presence of a soluble calcium salt, equal weights of the two modifications combine to form calcium racemate. Calcium racemate is very much less soluble than is either one of the calcium salts of its component parts; 100 grams of water at 25°C. dissolves 4.5 and 36.0 mg. of calcium racemate and calcium tartrate (laevo or dextro), respectively. The quantity of racemic acid formed will depend upon the quantity of tartaric acid

TABLE 3.

Added tartaric acid in apple jelly and blackberry jam.

| MATERIAL (30 GRAMS) | TARTARIC ACID ADDED | TARTARIC ACID DETERMINED | | ADDED TARTARIC ACID RECOVERED | TARTARIC ACID IN MATERIAL | |
|------------------------|------------------------|------------------------------|-------|-------------------------------------|------------------------------|------------------------|
| | mg. | mg. | | per cent | Present per cent | Determined per cent |
| Apple jelly | None | 2.3 2 0 | 2.2 | | | 0 007 |
| “ “ | 9 8 | 10 6 10 0 | 10.3 | 82 6 | 0 040 | 0.034 |
| “ “ | 73 9 | 73.8 72 5 | 73 2 | 96.0 | 0 254 | 0 244 |
| “ “ | 147 8 | 146 3 145 0 | 145 7 | 96 5 | 0 500 | 0 486 |
| Blackberry jam | None | 14 6 12 9 14.5 14 9 | 14.2 | | | 0 047 |
| “ “ | 10.1 | 22.5 23 0 | 22 8 | 85 1 | 0.081 | 0.076 |
| “ “ | 98 6 | 111 2 111 2 | 111.2 | 98.4 | 0 376 | 0 371 |
| “ “ | 197 2 | 209 5 210 6 | 210.1 | 99.3 | 0.705 | 0.700 |

present in the solution under examination. It is, of course, possible that the solution is entirely free of tartaric acid, in which case all the *l*-tartaric acid added will be converted into the difficultly soluble calcium salt. Obviously the quantity of *l*-tartaric acid used in the determination should be so adjusted that the excess calcium *l*-tartrate formed will remain in solution under all conditions of temperature and agitation. It was determined experimentally that 400 mg. of ammonium *l*-tartrate when treated as directed in the Nelson modification produces no precipitate when vigorously stirred and allowed to stand at 14°C. in the refrigerator overnight, and that 500 mg. under the same conditions gives a heavy precipitate. It is recognized that 14°C. is a lower temperature than would ordinarily be met with in the laboratory, nevertheless such a temperature may occur during the winter months. Accordingly, 400 mg. of ammonium *l*-tartrate was chosen as the maximum quantity to be used in the determination.

Concerning the oxidation of calcium racemate with potassium permanganate, it was found that the temperature at which the oxidation is conducted is of the greatest importance to the success of the determination. In Table 4 data are presented to show the effect which the temperature has upon the oxidation of tartaric acid.

TABLE 4.

*Effect of temperature on the oxidation of tartaric acid
with potassium permanganate.*

| TEMPERATURE AT WHICH OXIDATION WAS CONDUCTED. | TARTARIC ACID PRESENT | TARTARIC ACID DETERMINED |
|---|--------------------------|-----------------------------|
| °C. | mg. | mg. |
| 70 | 29.5 | 29.0 |
| 80 | 29.5 | 29.5 |
| 90 | 29.5 | 30.0 |
| 100 | 29.5 | 30.5 |
| 70 | 58.9 | 56.8 |
| 80 | 58.9 | 58.3 |
| 90 | 58.9 | 59.5 |
| 100 | 58.9 | 60.0 |
| 70 | 98.3 | 95.3 |
| 80 | 98.3 | 98.0 |
| 90 | 98.3 | 99.3 |
| 100 | 98.3 | 100.5 |
| 70 | 147.3 | 139.0 |
| 80 | 147.3 | 145.5 |
| 90 | 147.3 | 148.5 |
| 100 | 147.3 | 150.3 |

The results indicate that of the temperature used, 80°C. is more nearly the proper temperature for conducting the oxidation.

In Table 5 additional results on determinations on the oxidation of tartaric acid at 80°C. are presented.

TABLE 5.

*Oxidation of tartaric acid with potassium permanganate
at a temperature of 80°C.*

| TARTARIC ACID PRESENT | TARTARIC ACID DETERMINED |
|--------------------------|-----------------------------|
| mg. | mg. |
| | 20.3 |
| 20.0 | 20.5 |
| | 40.5 |
| 39.9 | 40.5 |
| | 60.5 |
| 59.9 | 60.3 |
| | 100.3 |
| 99.8 | 100.0 |
| | 199.5 |
| 199.6 | 199.5 |
| | 299.0 |
| 299.5 | 298.0 |

The results in Table 5 were obtained by using 150 cc. of the tartaric acid solution plus 50 cc. of a 10 per cent sulfuric acid solution and conducting the oxidation according to the procedure given in the proposed method.

Table 6 presents results obtained on the determination of calcium racemate.

TABLE 6.

Oxidation of calcium racemate with potassium permanganate at a temperature of 80°C.

| CALCIUM RACEMATE PRESENT $C_4H_4O_6Ca \cdot 4H_2O$ | RACEMIC ACID ANHYDROUS | RACEMIC ACID ANHYDROUS— DETERMINED |
|--|---------------------------|--|
| mg. | mg. | mg. |
| 24 2 | 14 0 | 14 4 |
| 57.5 | 33.2 | 33 5 |
| 105.2 | 60.7 | 60.5 |
| 182.5 | 105.3 | 103 9 |

The results of these experiments show that for accurate work it is necessary to conduct the oxidation at 80°C. Theoretically 400 mg. of ammonium *l*-tartrate will convert 325 mg. of tartaric acid into the racemic form; therefore, the solution under examination should not contain more than 325 mg. of tartaric acid. The procedure presented was formulated from the results of the foregoing experimentation.

DETERMINATION OF TARTARIC ACID AS CALCIUM RACEMATE.

PREPARATION OF SAMPLE.

Prepare the sample as previously directed in the paper for the determination of tartaric acid as acid potassium tartrate, following the directions there given through the filtration of the hydrogen sulfide precipitate (p. 105).

REAGENTS.

(a) *Di-ammonium citrate*.—Make a solution containing 50 grams to the liter. Dissolve 29.0 grams of citric acid in about 200 cc. of water and carefully neutralize with ammonium hydroxide, using methyl red as indicator. Add 14.5 grams of citric acid and make to 1 liter with water.

(b) *Ammonium l-tartrate*.—Dilute 3.2 grams entirely free from *d*-tartrate, to 200 cc. Add 1 cc. of formalin as a preservative.

(c) *Calcium acetate*.—Dissolve 16 grams of calcium carbonate in 120 cc. of glacial acetic acid diluted with sufficient water, make to 1 liter and filter.

(d) *Concentrated hydrochloric acid*.—Dilute 34 cc. to 1 liter.

(e) *Calcium carbonate*.—Dissolve 5 grams in 20 grams of acetic acid with 100 grams of sodium acetate, make to 1 liter and filter.

(f) *Potassium permanganate*.—Make a solution of 6.9745 grams per liter. Standardize this solution against a solution of pure tartaric acid of known titer, in the same manner as in the final titration. 1 cc. of the permanganate = nearly 0.005 gram of tartaric acid.

(g) *Oxalic acid*.—Make a solution containing 13.8793 grams per liter and titrate against the permanganate solution.

DETERMINATION.

Transfer 200 cc. of the clear filtrate obtained from the lead sulfide filtration to a 400 cc. beaker. Evaporate over a low flame to about 100 cc. to expel hydrogen sulfide, make to 150 cc. with water, and add 15 cc. of reagent (a), 25 cc. of reagent (b) and 20 cc. of reagent (c). Stir vigorously until calcium racemate begins to precipitate and allow to

stand overnight at room temperature. Filter by decantation onto a thin, tightly-tamped pad of asbestos in a Gooch crucible with removable bottom and transfer the precipitate to the Gooch with a portion of the filtrate. Wash the contents of the crucible five times with water, filling the crucible about half full and sucking dry each time. Treat the precipitate and mat, after removal from the Gooch, with 20 cc. of reagent (d) and wash the crucible thoroughly. Adjust the volume of the solution to 150 cc. with water, add 50 cc. of reagent (e), and bring to a temperature of 80°C. on the water bath. Cool the solution, stir vigorously, and allow to stand at least 4 hours, stirring occasionally. Filter, and wash as described in the first operation. Transfer the pad and precipitate to a casserole with 150 cc. of water, add 50 cc. of sulfuric acid, 10 per cent by volume, and heat to 80°C. Immediately add standard potassium permanganate solution until an excess is indicated. Again heat to 80°C., add an additional 5 cc. of the permanganate solution, and allow to stand about 1 minute. After reheating to 80°C., immediately add 10 cc. of the standard oxalic acid solution and titrate back with the permanganate solution. The weight of total tartaric acid ($d + l$), obtained by multiplying the number of cubic centimeters of potassium permanganate by 0.005, divided by 2 represents the weight of tartaric acid.

It will be noted that the general procedure for the precipitation of calcium racemate has not been changed from that proposed by Nelson. A more convenient method is offered for the preparation of the diammonium citrate. The quantity of ammonium *l*-tartrate added in the determination has been changed from 500 to 400 mg. The maximum quantity of total tartaric acid that can be present in the solution under examination is fixed at 200 mg. The precipitated calcium racemate is transferred to the Gooch with a portion of the filtrate. The temperature at which the oxidation is conducted has been changed from "near boiling" to 80°C.

This method was tried on solutions of citric and malic acids containing known quantities of tartaric acid.

TABLE 7.

Results on solutions containing tartaric, citric and malic acids.

| TARTARIC ACID PRESENT mg. | CITRIC ACID PRESENT mg. | MALIC ACID PRESENT mg. | TARTARIC ACID DETERMINED mg. | |
|---------------------------------|-------------------------------|------------------------------|------------------------------------|-------|
| 7.9 | 100 | 100 | 4.5 | 5.0 |
| | | | 5.5 | |
| 15.8 | 100 | 100 | 15.3 | 15.2 |
| | | | 15.0 | |
| 79.4 | 100 | 100 | 80.3 | 80.9 |
| | | | 81.5 | |
| 158.8 | 50 | 50 | 160.6 | 160.9 |
| | | | 161.1 | |
| 238.2 | 25 | 25 | 228.6 | 231.6 |
| | | | 234.5 | |

METHOD FOR THE APPROXIMATION OF TARTARIC ACID AS CALCIUM RACEMATE IN FRUITS AND FRUIT PRODUCTS.

Since in some instances extreme accuracy is not required, the following method for the determination of tartaric acid is presented. The removal

of pectin and the precipitation of the acid with lead are omitted. Use 200 cc. of a sample solution of a jam or jelly. In the case of fruit juices or products of similar nature use a quantity of the material which will contain not more than 200 mg. of acid calculated as tartaric acid. Adjust the volume to 150 cc. either by evaporation or the addition of water, and proceed directly according to the directions given for the determination of tartaric acid by the racemate method. Results obtained in this manner are contained in the last column of Table 8.

TABLE 8.

Comparison of methods for the determination of tartaric acid.

| MATERIAL | TARTARIC ACID ADDED | CALCIUM RACEMATE METHOD ON PRECIPITATED ACIDS PROPOSED | | ACID POTASSIUM TARTRATE METHOD, PROPOSED | | ACID POTASSIUM TARTRATE METHOD, OFFICIAL* | | CALCIUM RACEMATE METHOD DIRECT (APPROXIMATE) | |
|--|---------------------------|---|---------------|--|---------------|---|---------------|--|---------------|
| | | mg./100 grams | mg./100 grams | mg./100 grams | mg./100 grams | mg./100 grams | mg./100 grams | mg./100 grams | mg./100 grams |
| Concord grape juice—pure pasteurized | | 764 | | 736 | | 755 | | 779 | |
| | | 765 | | 728 | | 753 | | 780 | |
| | None | 755 | 761 | 726 | 730 | 752 | 753 | 781 | 780 |
| Apple jelly prepared in laboratory | | 335 | | 328 | | 350 | | 354 | |
| | | 337 | | 328 | | 355 | 353 | 352 | |
| | 333 | 337 | 336 | 335 | 330 | | | 356 | 354 |
| Raspberry jam— commercial | | 334 | | 317 | | 345 | | | |
| | | 329 | | 317 | | 333 | | 357 | |
| | 333 | 330 | 331 | 313 | 316 | 340 | 339 | 358 | 358 |

* Slight change in method of filtering the acid potassium tartrate. Blank determinations on apple jelly and raspberry jam by proposed acid potassium tartrate method, 0.007 per cent and 0.0 per cent, respectively.

In the determination of tartaric acid by the official method a slight change in the procedure was made. This change consisted in washing the acid potassium tartrate precipitate into the Gooch with the filtrate and then washing with the 20 cc. of wash solution. This was thought desirable since collaborators on the method have reported difficulty in removing foreign acids from the precipitated acid potassium tartrate with 20 cc. of the wash solution.

The outstanding feature in the comparison is the splendid checks obtained by all of the four methods. A comparison of the results obtained by the four methods on the materials in which the quantity of tartaric acid present is known are satisfactory for the two methods proposed. The high results obtained by the direct calcium racemate method are attributable to the presence of oxidizable material other than calcium racemate. Whereas in one instance the official method gives good results, in the other they are too high. Since the tartaric acid content of the grape juice is not known, the results cannot be interpreted as to the accuracy of the methods.

SUMMARY.

Two methods for the determination of tartaric acid in fruits and fruit products are proposed. Of these the acid potassium tartrate method is preferable to the calcium racemate method. The calcium racemate method requires a reagent that is expensive and not readily available, two precipitations are necessary, and the oxidation with potassium permanganate requires close attention. The acid potassium tartrate method requires less time, and the titration is easily accomplished. Particular attention is called to the necessity for removing pectin and isolating the acids precipitable with lead, as specified in both of the proposed methods. Following such a procedure no difficulty is experienced in the filtrations and titrations. The official method and the direct calcium racemate method, in which the pectin is not removed and the acids are not isolated, yield solutions which filter with difficulty, if at all.

DETERMINATION OF SILICA IN PHOSPHATE ROCK¹.

By W. L. HILL and K. D. JACOB (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.).

A lack of uniformity is apparent in the methods employed by different chemists for the determination of silica in phosphate rock. Frequently the material insoluble in concentrated hydrochloric acid or in aqua regia is reported as insoluble siliceous matter and considered to represent approximately the silica content of the rock. In some instances the residue insoluble in dilute acid is brought into solution by fusion with alkali carbonate, and the silica is determined by evaporation with hydrochloric acid without regard for any silica which may be dissolved by the initial acid treatment. A procedure in which the filtrate from the acid digestion of the sample is combined with the solution of the fused residue and the silica determined on the combined solutions is the method used in ordinary rock analysis; in the presence of notable amounts of fluorine, however, it is known to give low results, presumably due to volatilization of silicon as the tetrafluoride.

According to Stadeler² fluorine in amounts up to about 1 per cent has no appreciable effect on the silica as determined by the ordinary methods of rock analysis, but in the presence of more than 1 per cent of fluorine only the Berzelius method gives accurate results. Likewise, Guntz and Benoit³ obtained low results for silica in certain fluorine-bearing lithium minerals when the sample was subjected to direct acid treatment. Jacob and Reynolds⁴ have shown that all commercial types of domestic

¹ Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 28-30, 1929.

² *Stahl Eisen*, 47, 662-4 (1927).

³ *Bull. soc. chim.*, 37, 1294-7 (1925).

⁴ *This Journal*, 11, 237-50 (1928).

phosphate rock contain about 3 to 4 per cent fluorine. In view of this, low results for silica in phosphate rock may be expected from any method of analysis in which the rock sample is treated directly with acid, a fact which seems generally to have been overlooked in the analysis of natural phosphates. The literature contains no information relative to the actual losses of silica resulting from the formation of volatile fluorine compounds during the acid treatment in the analysis of phosphate rock.

In order to obtain complete and accurate chemical analyses of several samples of domestic types of phosphate rock, the authors were confronted with the tedious task of determining silica by the method of Berzelius. With the hope that by employing a uniform procedure a regular relationship could be found between the amount of fluorine present and the loss of silica, whereby the necessity for using the Berzelius method might be eliminated, the silica was determined on a number of samples both by the Berzelius method and by the method used in ordinary rock analysis.

Although the results obtained give no indication of any simple relationship between the loss of silica and any other one factor, they are of interest from an analytical point of view in that they give a qualitative measure of the error in results obtained on phosphate rock by ordinary methods of rock analysis. Furthermore, in view of the increasing demand for greater accuracy in the analysis of phosphate rock, it seemed appropriate at this time to present data for the determination of silica, both by a modified form of the Berzelius method and by the ordinary method, on five commercial types of domestic phosphate rock. Two samples of Florida soft phosphate were included since this material promises to be of commercial importance.

ANALYTICAL METHODS.

For the determination of silica without regard for fluorine, the method for the analysis of silicate rocks described by Hillebrand¹ was used on 2.5 grams of the rock powder with the following modifications: (1) The sample was digested 30 minutes with 50 cc. of dilute hydrochloric acid (1 + 1) in platinum on the boiling water bath; (2) the filtrate from the initial acid treatment was evaporated to dryness before adding the solution obtained by treating the fused residue with acid, because slightly higher silica values were obtained thereby and also the time required for the determination was shortened considerably; (3) the silica remaining in the filtrate after the second evaporation with acid was not recovered. For the sake of brevity this procedure will be called the ordinary method. The weight of the residue from the initial acid

¹ U. S. Geological Survey Bull. 700, pp. 94-104 (1919).

digestion, after being ignited to constant weight at 1000°C., is reported as insoluble material.

The procedure described by Hillebrand¹ was followed in the few determinations made by the Berzelius method. The modified Berzelius method, developed by Hoffman and Lundell² for the determination of fluorine and silica in glass, has the advantage of being shorter and easier to carry out. In the latter procedure 1 gram samples were used.

All samples were ground to pass a 100-mesh sieve. Blank determinations were carried through all operations, and the proper corrections were applied to obtain the final values for silica. In the modified Berzelius method the silica recovered by precipitation with ammonia and subsequent evaporation with sulfuric acid amounted to about 1 mg.

RESULTS AND DISCUSSION.

The results given in the tables are the mean of duplicate determinations agreeing within less than 0.1 per cent, usually less than 0.05 per cent, for the insoluble material and the silica by the ordinary method. With the Berzelius procedure and its modification the disparity in the results of duplicate determinations reached a maximum of 0.15 per cent, but usually it was 0.1 per cent or less.

TABLE 1.

Effect of time of initial digestion with acid in the ordinary method.

| SAMPLE NO. | INSOLUBLE IN DILUTE HCl (1+1) | | | SILICA | | | TYPE OF PHOSPHATE |
|------------|-------------------------------|------------------|-----------------|------------------|------------------|-----------------|----------------------|
| | Digested 30 min. | Digested 60 min. | Difference | Digested 30 min. | Digested 60 min. | Difference | |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | |
| 439 | 6.59 | 6.42 | 0.17 | 6.30 | 6.24 | 0.06 | Florida pebble |
| 618 | 8.55 | 8.27 | 0.28 | 8.14 | 8.14 | 0.00 | " " |
| 619 | 6.84 | 6.62 | 0.22 | 6.52 | 6.49 | 0.03 | " " |
| 906 | 5.71 | 5.22 | 0.49 | 5.02 | 4.86 | 0.16 | Tennessee brown rock |

The results given in Table 1 show the effect of the time of the initial acid digestion on the insoluble material and the silica recovered by the ordinary method. It is of interest to note that while the longer period of digestion of the Florida pebble lowered the insoluble material 0.2–0.3 per cent, it had little effect on the silica. In the Tennessee brown rock the insoluble was lowered 0.5 per cent and the silica 0.16 per cent. This difference in the behavior of the two types of rock when treated with

¹ U. S. Geological Survey Bull. 700, p. 226.

² *Bur. Standards J. Res.*, 3, 581-95 (1929); Cf. Hillebrand and Lundell, *Applied Inorganic Analysis*, p. 805. John Wiley & Sons, New York, 1929.

dilute acid was most likely due to the state in which the silica existed in the rock. The silica is known to exist principally as coarse grains of quartz in Florida pebble, while in Tennessee brown rock most of it is present in a very finely divided state.

TABLE 2.

Comparison of the Berzelius with the modified Berzelius method.

| SAMPLE NO. | SILICA | | TYPE OF PHOSPHATE |
|------------|------------------|---------------------------|---------------------|
| | Berzelius Method | Modified Berzelius Method | |
| | <i>per cent</i> | <i>per cent</i> | |
| 771 | 12.95 | 13.02 | Florida hard rock |
| 772 | 9.80 | 9.71 | Tennessee blue rock |
| 773 | 8.15 | 8.15 | Idaho rock |

Results obtained by the Berzelius and by the modified Berzelius methods are in good agreement, as indicated in Table 2. The modified method seems to be as accurate as the older procedure, and it is to be preferred on account of its greater speed and ease of performance.

In Table 3 the "Loss of Silica" is the difference between the results found by the modified Berzelius and by the ordinary method. The maximum amount of silica which could be lost in the event that all the fluorine were volatilized as silicon tetrafluoride is given in the seventh column.

Before these results are discussed it should be noted that the analysis of a synthetic mixture is included at the end of Table 3. This mixture, prepared from quartz and tricalcium phosphate of known purity and Bureau of Standards fluorspar, standard sample No. 79, contained 8.83 per cent silicon dioxide, as calculated from the analyses of the individual materials. However, upon analysis of the mixture only 8.60 per cent silicon dioxide was recovered by the modified Berzelius method, a loss of 0.23 per cent, which indicates that values obtained by this method may be somewhat low.

When the presence of fluorine was disregarded in the analysis, in every case the silica values were low. An idea of the amount of silica lost may be obtained from the results given in the sixth column of Table 3. The figures range from about 0.5 to 2.7 per cent of the sample and vary with the type of rock as well as with samples of the same rock type taken from different deposits. The Florida pebble falls into two groups. In the upper group the difference between the silica as determined by the two methods varies from 2.1 to 2.7 per cent, in the lower from 1.4 to 1.7 per cent. In the Florida hard rock the variation is from

TABLE 3.

Comparison of modified Berzelius method with the ordinary method.

| SAMPLE NO. | TOTAL* FLUORINE | INSOLUBLE IN DILUTE HCl (1 + 1) | SILICA | | LOSS OF SILICA | SiO ₂ EQUIVALENT TO ALL F AS SiF ₄ | LOCATION OF PHOSPHATE DEPOSIT |
|--------------------------------|-----------------|---------------------------------|-----------------|---------------------------|-----------------|--|---|
| | | | Ordinary Method | Modified Berzelius Method | | | |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | |
| FLORIDA PEBBLE PHOSPHATE | | | | | | | |
| 439 | 3.87 | 6.59 | 6.24 | 8.32 | 2.08 | 3.07 | Mulberry |
| 617 | 4.01 | 6.98 | 6.81 | 9.02 | 2.21 | 3.17 | Brewster |
| 618 | 3.92 | 8.55 | 8.14 | 10.71 | 2.57 | 3.10 | Pierce |
| 619 | 3.98 | 6.84 | 6.49 | 9.18 | 2.69 | 3.15 | Nichols |
| 622 | 3.96 | 7.07 | 6.72 | 8.39 | 1.67 | 3.14 | " |
| 627 | 3.90 | 7.29 | 6.98 | 8.58 | 1.60 | 3.08 | Lakeland |
| 790 | 3.97 | 7.28 | 7.07 | 8.92 | 1.85 | 3.14 | Unknown |
| 898 | 3.95 | 6.29 | 5.98 | 7.38 | 1.40 | 3.12 | Lakeland |
| FLORIDA HARD-ROCK PHOSPHATE | | | | | | | |
| 434 | 3.76 | 3.62 | 3.35 | 4.32 | 0.97 | 2.97 | Dunnellon |
| 589 | 3.79 | 5.60 | 5.44 | 6.39 | 0.95 | 3.00 | Floral City |
| 771 | 3.35 | 11.68 | 12.54 | 13.02 | 0.48 | 2.65 | Unknown |
| FLORIDA SOFT PHOSPHATE | | | | | | | |
| 726 | 1.81 | 20.02 | 18.10 | 19.78 | 1.68 | 1.43 | Juliette |
| 728 | 3.33 | 9.67 | 9.80 | 12.40 | 2.60 | 2.63 | Hernando |
| TENNESSEE BROWN-ROCK PHOSPHATE | | | | | | | |
| ... | 3.56 | 8.93 | 6.98 | 9.26 | 2.28 | 2.81 | Bureau of Standards, Standard Sample No. 56 |
| 564 | 3.62 | 7.55 | 6.85 | 8.12 | 1.27 | 2.86 | Wales |
| 587 | 3.24 | 14.38 | 13.07 | 14.14 | 1.07 | 2.56 | " |
| 762 | 3.87 | 5.53 | 5.28 | 7.49 | 2.21 | 3.07 | Mt. Pleasant |
| 905 | 3.75 | 5.71 | 5.02 | 7.11 | 2.09 | 2.96 | Wales |
| TENNESSEE BLUE-ROCK PHOSPHATE | | | | | | | |
| 448 | 3.67 | 8.24 | 7.09 | 7.68 | 0.59 | 2.90 | Gordonsburg |
| 449 | 3.95 | 6.26 | 3.98 | 11.89 | 0.91 | 3.12 | " |
| 772 | 3.49 | 9.52 | 7.81 | 9.80 | 1.99 | 2.76 | Glover |
| IDAHO PHOSPHATE | | | | | | | |
| 454 | 3.40 | 6.47 | 5.77 | 7.50 | 1.73 | 2.69 | Conda |
| 550 | 3.43 | 10.60 | 9.53 | 10.36 | 0.83 | 2.71 | Paris |
| 773 | 3.36 | 6.66 | 6.01 | 8.15 | 2.14 | 2.66 | Conda |
| SYNTHETIC MIXTURE | | | | | | | |
| . | 4.01 | | 8.05 | 8.60 | 0.55 | 3.16 | |

* With the exception of Sample No. 906, the figures for fluorine are taken from a paper by Reynolds, Jacob and Hill, *Ind. Eng. Chem.*, 21, 1253-8 (1929).

0.5 to 1.0 per cent. With the other types the variations are between somewhat wider limits and appear irregular, although samples from the same locality agree, in general, fairly well.

It is evident that the loss of silica resulting from analysis when the ordinary method is used bears no definite relationship to the fluorine content of the rock. Although the total fluorine was practically con-

stant in all the samples of Florida pebble analyzed, the amounts of silica lost varied between relatively wide limits. Similar variations were found in the other types of rock.

The results show that, with one exception, the loss of silica is less than what it should be if all the fluorine were volatilized as silicon tetrafluoride. This is to be expected since silicon tetrafluoride is unstable in the presence of moisture, and some of the fluorine probably escapes as hydrogen fluoride while a part is fixed as fluosilicates. The exception is No. 726, in which the loss of silica was appreciably greater than the amount which could be volatilized by the fluorine present. At present the writers are unable to explain this irregularity.

The quantity of acid-insoluble material, as a rule, was considerably less than the total silica, as determined by the modified Berzelius method. Thus, in Florida pebble the differences range from 1.1 to 2.2 per cent, in Florida hard-rock from 0.7 to 1.3 per cent, and in Tennessee brown-rock from 0.3 to 2.0 per cent. Tennessee blue-rock is an important exception to this rule. This type is known to contain large quantities of iron pyrites, and for this reason a relatively large amount of insoluble material in addition to the siliceous matter was to be expected.

SUMMARY.

Results are given showing the effect that length of time of the acid digestion of the sample has on the results for the insoluble material and silica in phosphate rock as determined by the ordinary method.

The Berzelius method for the determination of total silica in the presence of fluorine and a modification of this method were compared, and the modified method is shown to be applicable to the determination of silica in phosphate rock.

Silica was determined in a number of samples of phosphate rock by the modified Berzelius and the ordinary methods. The results show that, owing to the presence of fluorine in phosphate rock, the percentages of silica obtained by methods of ordinary rock analysis are 0.5–2.7 per cent lower than the percentages obtained by the modified Berzelius method.

THE ASSAY OF RESIN OF PODOPHYLLUM*.

By L. E. WARREN (Drug Research Unit, Food, Drug and Insecticide Administration, Washington, D. C.).

The purgative properties of the mayapple, *Podophyllum Pellatum*, were known to the pre-Columbian Indians of North America, according to Griffith¹. The early white settlers learned of the medicinal value of the plant from the natives. The discovery of resin of podophyllum is claimed

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¹ Medical Botany, 116 (1847).

to have been made by Dr. John King, an eclectic physician in 1835¹, although he did not publish his claims until 1844. He obtained the resin by making a tincture of the drug, evaporating most of the alcohol, adding water and cooling the concentrate. On standing the resin separated in flakes. That the drug contains resin, however, had been recorded by Rafinesque² earlier than this. He says that the drug "contains resin, fecula, bitter extractive, gallic acid and a gummy substance". However, the writer of this paper searched the available published writings of Rafinesque, but he did not find any recorded analyses of podophyllum made by him nor any references to the sources of his information.

In 1847 Lewis³ prepared a solid extract of the drug by maceration with alcohol and subsequent evaporation. This extract was only partly soluble in ether. The ether-insoluble portion was boiled with water, the insoluble portion was dissolved in alcohol, the solution was treated with animal charcoal, and the filtrate was allowed to evaporate spontaneously. The residue, after being washed with water, acted as a drastic cathartic in 6 grain doses.

Smith⁴ appears to have been the first pharmacist to prepare the resin by pouring a concentrated extract of the drug into a large volume of water. This method is essentially that now described in the U. S. Pharmacopeia, except that a small quantity of hydrochloric acid is now contained in the precipitating medium.

The name, "resin of podophyllum", is a misnomer since the product is not a true resin but a mixture of substances. It has been established that resin of podophyllum consists chiefly of podophyllotoxin, a crystalline substance having purgative properties, phodophyllo-resin, which has purgative properties, and quercetin, a coloring substance. Small but variable quantities of reducing sugars and moisture are usually present as impurities. Some brands of the commercial article may contain small amounts of aluminum salts, impurities resulting from the use of alum as a precipitant.

The statement is sometimes made that resin of podophyllum contains, among other things, two resins, one soluble in ether and physiologically active and the other insoluble in ether and inert. The assertion evidently harks back to the studies made by Cadbury⁵ in 1858. He prepared the resin from the drug and treated it with ether. The insoluble portion was dissolved in hot alcohol, and the solution was boiled with animal charcoal until colorless. After filtration and evaporation the residue was physiologically inert in doses of one grain. The ether-soluble fraction (which was not treated with charcoal) was active in doses of one-fourth

¹ N. Y. Philosoph. Med. Journ., 1, 157 (1844).

² Med. Flora, U. S., 2, 60 (1830).

³ Am. J. Pharm., 19, 169 (1847).

⁴ Ibid., 24, 306 (1852).

⁵ Ibid., 30, 301 (1858).

grain. Cadbury did not make any tests on the ether-insoluble fractions of the resin that had not undergone further treatment. The assertion has been made that Power verified these findings, but he is not accurately quoted. Power¹ prepared the resin in the usual way and evaporated the mother liquor. By this procedure there was obtained a second crop of resin, which he subjected to ether extraction, thereby separating it into two portions—one soluble and the other insoluble. The insoluble part was only feebly physiologically active, while the ether-soluble portion was very active. Power apparently assumed that the second crop of resin was identical with the first—an assumption by no means warranted. He states, however, that by a series of experiments, the details of which he does not give, he had confirmed his earlier findings, viz., that although the ether-insoluble fraction was not without some activity the ether-soluble portion was much more active.

Dunstan and Henry² exhausted resin of podophyllum with chloroform in a Soxhlet apparatus and extracted the insoluble residue with ether. The residue still remaining had the properties of a resin and was physiologically active. They called this substance *podophyllo-resin*.

Several methods for the assay of resin of podophyllum have been proposed. The Eder and Schnceiter method³, which is an adaptation and elaboration of the processes worked on by Podwissotzki⁴, Kremel⁵, Kürsten⁶, and others⁷, is generally considered the best. In this the podophyllotoxin is isolated and weighed. The methods of Gordin and Merrell⁸ and that of Umney⁹ are generally regarded as too long and tedious for practical purposes. In these processes the podophyllotoxin is converted into its isomer, picropodophyllin, which is weighed. In 1914 Jenkins¹⁰ published a method for the assay of podophyllum and its preparations in which the resin was isolated and weighed. In a trial test he applied the method to one specimen of the isolated resin from podophyllum and recovered 98.4 per cent of the amount taken.

In 1916 Tanzen¹¹ reported that he had assayed twelve specimens of resin of podophyllum of American origin for podophyllotoxin, each by five methods. From his results he concluded that the method of the Dutch Pharmacopeia (official at that time) was the best. One of the methods used by Tanzen was the Jenkins process for the assay of podophyllum. Apparently Tanzen assumed that the method yields podophyllotoxin instead of total resin, a suggestion never made by Jenkins.

¹ *Am. J. Pharm.*, 46, 227 (1874).

² *J. Chem. Soc.*, 73, 221 (1898).

³ *Pharm. Acta Helv.*, 1, 15 (1926).

⁴ *Pharm. Z. Russland*, 20, 208, 777, 793, 809, 825, 841, 861 and 882 (1881).

⁵ *Pharm. Post*, 22, 105 (1889).

⁶ *Arch. Pharm.*, 229, 220 (1891).

⁷ *Pharm. Nederland V.*

⁸ *Proc. Am. Pharm. Assoc.*, 50, 343 (1902).

⁹ *Pharm. J.*, 87, 156 (1911).

¹⁰ Jenkins, W. M.: *J. Ind. Chem.*, 6, 671 (1914).

¹¹ Tanzen, H.: *Arch. Pharm.*, 254, 44 (1916).

Tanzen reports finding from 35 to 46.5 per cent of podophyllotoxin in the twelve specimens of resin by the Jenkins process. The writer has been unable to verify Tanzen's findings by the Jenkins process, and he cannot understand how such results could have been obtained. The writer's findings were 93.5 and 91.2 per cent, respectively, on a commercial brand of resin. Apparently Eder and Schneider¹ also fall into the same error as Tanzen in assuming that the Jenkins process gives podophyllotoxin. They criticize the method because it requires a large amount of sample and because the process is somewhat complicated. Although they state that the method gives higher results than others, they did not report any results obtained by them by the method.

Several purchasers of resin of podophyllum report² that the market supplies of the product are not uniform in composition. Their judgments were based on results obtained by subjecting the purchased material to the tests described in U. S. Pharmacopeia X. The Pharmacopeia requires that resin of podophyllum shall not yield more than 1.5 per cent of ash, shall be practically soluble in alcohol, and shall contain not less than 75 per cent of ether-soluble material and not less than 65 per cent of chloroform-soluble substance. An exclusion test for resin of Indian podophyllum is also provided.

Some purveyors of resin of podophyllum, as well as certain manufacturers of the article², believe that the "ether-soluble" and "chloroform-soluble" tests of the Pharmacopeia are empiric only, and that the results obtained by the employment of these tests add little to the knowledge of the quality of the product,—in other words they believe that the insoluble portions of the resin possess some physiological activity, and that consequently the determination of the soluble portions of the resin by these solvents does not constitute a true assay. Several analysts reported³ that in the U. S. P. test for "ether-soluble" material the quantity of ether specified to be used for 1 gram of the resin (20 cc.) is inadequate to dissolve out all the ether-soluble fractions from that quantity of resin. Some of them have pointed out that the U. S. P. IX method, which did not restrict the amount of solvent, was satisfactory. Consequently these analysts now use 30 cc. or more of ether for the test. One manufacturer believes that the ether solubility of various lots of the resin depend to some extent on the moisture content of the material. He suggested that the moisture be determined in a separate portion and the "ether-soluble" determination be then made on the dried material. The results should then be calculated in terms of the moisture-containing market product. Another manufacturer found that it was essential to employ ether of U. S. P. quality in making the ether-solubility test.

¹ Loc. cit.

² Jenkins, W. M.: *J. Ind. Chem.*, 6, 671 (1914).

³ Private communications to the writer.

This may contain from 2 to 4 per cent of a mixture of alcohol and water. When anesthetic ether was used the results were always low.

This study was undertaken with the view to obtaining more information concerning the reliability of the tests for resin of podophyllum that are described in the U. S. Pharmacopeia. Secondary objectives were to make comparative studies of the several methods that have been proposed for the assay of the resin and to ascertain if possible which of them gives the truest evaluation of the quality of the drug.

In order to study the Pharmacopeial tests it was decided to apply them to several specimens of resin of podophyllum. Accordingly nine brands of the resin were obtained from the original manufacturers and one specimen was prepared in the laboratory from authenticated drug according to the directions of U. S. P. X. The ash, "ether-soluble", and "chloroform-soluble" were determined according to the methods described in U. S. P. X, except that the sample taken for incineration was approximately 1 gram instead of from 2 to 4 grams, and the initial quantity of ether taken for the "ether-soluble" test was 30 cc. instead of 20 cc. Moisture was determined by loss over sulfuric acid and loss at 65°C. "Alcohol-insoluble" material was determined by the following method:

ALCOHOL-INSOLUBLE.

Weigh 2 grams of the powdered material into a beaker of about 150 cc. capacity. Add 50 cc. of alcohol and agitate gently for a few minutes. Decant the liquid through a weighed Gooch crucible and wash the container with several small portions of alcohol, taking care to transfer any insoluble material to the crucible. Wash the crucible several times with small portions of alcohol. If difficulty is experienced in washing the crucible contents free from alcohol-soluble substances, place the partially-washed crucible and contents in a Bailey extractor¹ and extract with alcohol to exhaustion. Dry the crucible to constant weight at 100°C. and weigh.

The results for the ten specimens may be found in Table 1.

An examination of the results given in Table 1 shows that those for "ether-soluble" and "chloroform-soluble" are erratic. In some specimens good duplicates were obtained, while in others it was impossible to get results that would agree within several per cent. It was noted that in general the specimens that gave good duplicate results with ether also gave similar results with chloroform and *vice versa*. Only one specimen conformed to the U. S. P. requirements in respect to solubilities in both solvents. In view of the fact that so many criticisms concerning these two tests had been received, it seemed worth while to inquire whether they are of any value as assay processes, i. e. in separating the resin into factions which are respectively active and inert. As before mentioned, the studies of Lewis and Power indicated that the ether-insoluble part of the resin possessed some activity, and Dunstan and Henry showed

¹ *J. Ind. Eng. Chem.*, 6, 497 (1914).

that the portion insoluble both in chloroform and ether was active. However, these investigators did not report the relative activity of this residue as compared with the original resin.

TABLE 1.
Analysis of resin of podophyllum.

| SPECIMENS | LOSS OVER H ₂ SO ₄ | LOSS AT 65°C. | ASH | ETHER- SOLUBLE | CHLORO- FORM- SOLUBLE | ALCOHOL- INSOLUBLE | TOTAL RESINS | PODOPHYLL- LOTOXIN |
|-------------------------------|--|---------------------|-----------------|-------------------|-----------------------------|-----------------------|-----------------|-----------------------|
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| A | 2.25 | 3.33 | 0.42 | 71.22 | 60.68 | 0.99 | 89.26 | 46.69 |
| | 2.23 | 3.42 | 0.42 | 71.21 | 58.20 | 1.09 | 89.20 | 46.26 |
| | | | | | 60.67 | 1.34 | 89.50 | 46.50 |
| B | 2.70 | 2.97 | 0.61 | 70.03 | 62.13 | | 87.93 | 46.95 |
| | 2.72 | 3.05 | 0.53 | 69.86 | 62.72 | 1.60 | 86.16 | 47.22 |
| | | | | | 61.47 | 1.49 | 90.85 | |
| C | | 2.33 | 0.33 | 73.63 | 61.80 | 0.50 | 96.0 | 47.35 |
| | | 2.35 | 0.28 | 73.72 | 62.42 | 0.49 | 94.3 | 47.15 |
| | 2.32 | | | | 63.12 | 0.46 | 95.8 | |
| | 2.31 | | | | 62.66 | 0.66* | 96.5* | |
| D | 2.17 | 2.74 | 1.16 | 69.83 | 58.73 | 1.04 | 95.2 | 46.26 |
| | 2.00 | 2.86 | 1.08 | 70.40 | 57.53 | 0.61 | 96.3 | 46.40 |
| | | | 1.03* | 68.32 | 65.10* | 0.65 | 96.4 | |
| | | | | 80.5* | | | 96.1 | |
| E | 2.00 | 2.48 | 0.19 | 70.88 | 64.80 | 0.25 | 93.26 | 45.60 |
| | 1.84 | 2.52 | 0.19 | 66.48 | 64.77 | 0.19 | 92.45 | 45.51 |
| | | | 0.22* | 69.73 | 68.34* | 1.00* | 93.00 | |
| | | | | 76.15* | | | | |
| F | 1.82 | 3.54 | 0.34 | 90.31 | 73.82 | 1.26 | 92.80 | 52.56 |
| | 1.79 | 3.21 | 0.36 | 87.20 | 68.78 | | 94.25 | 52.65 |
| | | | | 77.93 | 68.97 | | 93.40 | |
| G | 1.89 | 2.87 | 0.32 | 72.96 | 70.49 | 0.22 | 94.60 | 49.37 |
| | 1.97 | 2.80 | 0.28 | 74.52 | 70.37 | 0.25 | 94.95 | 49.77 |
| | | | 0.35* | 74.44 | | | 95.15 | 49.87 |
| H† | 1.84 | 1.83 | 0.55 | 61.19 | 54.93 | 1.88 | 85.80 | 42.72 |
| | 1.82 | 1.74 | 0.55 | 49.76 | 54.34 | | 86.45 | 43.12 |
| | | | | 54.05 | | | 86.10 | |
| I | 1.65 | 1.73 | 0.29 | 69.62 | 63.97 | 1.08 | 95.60 | 47.68 |
| | 1.73 | 1.72 | 0.29 | 69.75 | 63.77 | | 95.10 | 47.27 |
| | | | | 75.95* | 67.40* | | 95.45 | 47.74 |
| | | | | 77.45* | 66.70* | | | |
| J Labora- tory- made | 2.46 | 2.90 | 0.16 | 58.14 | 60.68 | 0.21 | 93.75 | 46.15 |
| | 2.56 | 2.97 | 0.17 | 60.07 | 60.67 | | 93.75 | 45.97 |
| | | | | 60.02 | | | | |

* Reported by manufacturer.

† Stated to have been made by a process different from that of the U. S. P.

In order to obtain more information on these points, tests were made on the laboratory specimen of resin previously mentioned. The portions respectively insoluble in ether and chloroform were prepared by repeatedly macerating portions of the resin with the solvent and washing the insoluble resin with fresh portions of the solvent. The insoluble fractions were dried in the air. Portions of the original resin and the fractions respectively insoluble in ether and chloroform were mixed with lactose in the proportion of 1 to 5. The threshold dose (that is, the smallest dose of the mixture which would produce one extra bowel movement per day or a movement softer than normal) was then determined approximately by administration to a healthy man. Likewise the same values were determined in the Pharmacological Laboratory for cats. The results are given in Table 2.

TABLE 2.
Activity of resin on podophyllum.
(Smallest effective dose.)

| | NORMAL RESIN | ETHER- INSOLUBLE | CHLOROFORM- INSOLUBLE | ETHER- AND CHLOROFORM- INSOLUBLE |
|--------------------------|---|---------------------|--------------------------|--|
| | <i>gram</i> | <i>gram</i> | <i>gram</i> | <i>gram</i> |
| Man weighing 80 K. | 0.0108 (1/6 gr.) 0.0081 (1/8 gr.) | 0.0243 (3/8 gr.) | 0.0162 (1/4 gr.) | 0.0324 (1/2 gr.) |
| Cat weighing 3 K. | 0.0054 (1/12 gr.) 0.00405 (1/16 gr.) | 0.0081 (1/8 gr.) | 0.0108 (1/6 gr.) | 0.0108 (1/6 gr.) |

The experiments listed in Table 2 demonstrate that the ether-insoluble fraction and the chloroform-insoluble fraction of the resin are each quite active, but that in equivalent weights neither is as active as the original resin. It is believed that the evidence obtained in these experiments coupled with that obtained in the solubility tests warrants the conclusion that the ether-solubility test and the chloroform-solubility test for resin of podophyllum are of little importance so far as evaluating the drug is concerned. If the tests are to be used at all the technic described in U. S. P. X should be modified so as to employ an excess of solvent, the losses caused by scanty washing on the filter being thereby reduced. These tests appear to have been first used by Gordin and Merrell, but they employed an excess of solvent.

As a result of some experimentation, a method was devised for determining the "ether-soluble" and the "chloroform-soluble" portions of the resin, and it appeared to give more satisfactory results than those obtained by the U. S. P. process. As modified the method is as follows:

ETHER-SOLUBLE.

Weigh 3 grams of the resin into a 200 cc. glass-stoppered Erlenmeyer flask and add 50 cc. of ether. Shake gently by rotation at intervals for 30 minutes. Decant the supernatant liquid through a 11 cm. filter into a 100 cc. graduated flask and wash the insoluble residue with 20 cc. of ether. Allow the residue on the filter to dry spontaneously and transfer as much of the insoluble material as convenient to the original container. Break up any lumps with a flat-headed glass rod, add 30 cc. of ether, and shake the mixture gently at intervals for 15 minutes. Decant the mixture through the original filter, collecting the filtrate in the graduated flask. Wash the insoluble material with small portions of ether until the washings become colorless, concentrate the washings if necessary by evaporation, and add the solution to the graduated flask. Cool the solution and make up to 100 cc. with ether. Evaporate an aliquot portion of 10 cc. of the solution in a weighed dish, taking care near the end of the evaporation to rotate the container in an inclined position, and dry the residue to constant weight at 80°C.

CHLOROFORM-SOLUBLE.

Employ the method described above, using chloroform as the solvent.

It has been suggested that the ether solubility test and the chloroform solubility test might be of value in excluding adulterants. This suggestion is not logical because most other resins are more soluble in ether and in chloroform than is resin of podophyllum. This may be seen by examining Table 3 in which the solubility of several resins which are used in medicine are tabulated. Resin of podophyllum is included for comparison.

TABLE 3.

Solubility of several resins in ether and chloroform.

| RESIN | ETHER | CHLOROFORM |
|-------------|-----------------|-----------------|
| Guaiac | Soluble | Soluble |
| Ipomea | 80-90% | Soluble |
| Jalap | Not over 12% | Not over 30% |
| Mastiche | At least 97% | Soluble |
| Podophyllum | At least 75% | At least 65% |
| Rosin | Soluble | Soluble |

From an examination of the table it may be seen that the only resin that would arouse suspicion because of its lesser solubility in ether and chloroform is jalap. Since the price of resin of jalap is usually higher than resin of podophyllum its use as an adulterant of the latter is not likely.

It has been asserted that the value of resin of podophyllum is accurately estimated by the percentage of podophyllotoxin that it yields¹. Therefore some experiments were made with a view to assaying the resin for podophyllotoxin. Examination of the literature revealed that the method given in the new Dutch Pharmacopeia, as modified by Eder and Schneider², is considered the best. After some experimentation the writer still further modified the method. As used here the method is as follows:

PODOPHYLLOTOXIN.

Weigh 3 grams of the resin into a 200 cc. glass-stoppered Erlenmeyer flask and add 50 cc. of chloroform. Shake gently by rotation at intervals for 30 minutes. Decant the supernatant liquid through a 11 cm. filter into a 100 cc. graduated flask and wash the insoluble residue with 20 cc. of chloroform. Allow the residue on the filter to dry spontaneously and transfer as much of the insoluble material as convenient to the original container. Break up any lumps with a flat-headed glass rod, add 30 cc. of chloroform, and shake the mixture gently at intervals for 15 minutes. Decant the mixture through the original filter, collecting the filtrate in the graduated flask. Wash the insoluble material with small portions of chloroform until the washings become colorless, concentrate the solution if necessary by evaporation, and add the residue to the graduated flask. Cool the solution and make up to 100 cc. with chloroform.

Pour 10 cc. of the solution into 50 grams (80 cc.) of petroleum benzin contained in a weighed Erlenmeyer flask. As soon as the precipitate has subsided filter through a weighed Gooch crucible and wash the precipitate and flask with 20 cc. of petroleum benzin. Dry the crucible and flask for an hour at 70°C. Allow them to stand in the desiccator for an hour and weigh. Add the weight of material adhering to the flask to the weight of material in the crucible.

The findings for the ten specimens are found in Table 1. All the results fall between 45 and 50 per cent of podophyllotoxin with the exception of those for one specimen (F). Owing to shortage of material, the assays on this specimen were made on material that had been dried over sulfuric acid. Although the findings were calculated in terms of the undried resin, it is reasonable to suppose that the dried material would yield more soluble material to chloroform than the undried product with a resultant increase in the yield of podophyllotoxin.

In order to ascertain whether this method removes all the active ingredients of the resin (which are soluble in chloroform) the filtrate from the determinations of podophyllotoxin was evaporated to dryness on the steam bath. The residue was triturated with lactose, and portions of the mixture were administered to cats. The residue was found to be very active. Since previous tests had demonstrated that the chloroform-insoluble fraction of the resin was physiologically active and this test proves that the assay of podophyllotoxin does not remove all the active ingredients in the chloroform-soluble fraction, it is evident that the assay for podophyllotoxin is not a reliable index on which to judge

¹ U. S. Disp., 20, 945 (1918).

² *Loc. cit.*

the quality of the resin, although in an examination of several specimens it may have comparative value.

After a careful examination of the literature and some experimental trials it seemed inadvisable to consider any of the other methods proposed for the assay of resin podophyllum except the one suggested by Jenkins¹. After numerous trials several modifications of the original method were made. As finally used the method is as follows:

Transfer the filtrate from the determination of alcohol-insoluble material (as previously described) to a 100 cc. graduated flask and make the solution up to volume with alcohol. Transfer 10 cc. of the solution, equivalent to 0.2 gram of resin of podophyllum, to a separator by means of a pipet, and add 10 cc. of chloroform and 10 cc. of 0.6 per cent hydrochloric acid (2 cc. of hydrochloric acid in 100 cc. of water). Shake the mixture and allow it to separate, draw off the lower layer into another separator, and repeat the extraction of the liquid in the first separator three times, using each time 15 cc. of a mixture of one volume of alcohol and two volumes of chloroform, and adding these extractions to the extractive in the second separator. Shake the combined extractions with 10 cc. of 0.6 per cent hydrochloric acid and allow the mixture to separate. Draw off the lower layer into a weighed flask, and repeat the extraction of the acid liquid three times, using 15 cc. of fresh alcohol-chloroform mixture each time. Evaporate the combined alcohol-chloroform extractions, taking care near the end of the evaporation to rotate the container in an inclined position, and dry the residue to constant weight at 80°C.

The findings for the several specimens are recorded in Table 1. The results vary from about 85 to over 96 per cent. In view of this wide variation it seemed worth while to ascertain whether all the physiologically active resins were removed by the extractive process. To gain information on this point the acid residues remaining in the separators from 6 assays were made slightly alkaline with ammonia water, the liquids were united, and the solution was evaporated to dryness on the steam bath. The residue was then macerated with absolute alcohol slightly acidified with hydrochloric acid. The mixture was filtered, and the filtrate was evaporated to dryness and weighed. The residue was mixed with four times its weight of lactose, and the mixture was administered to cats in 1/8 grain doses. It was inactive. In view of the relatively large amount of material represented by this test it is considered that the assay process removes practically all the active ingredients from the drug. The method therefore appears to give a better index of the therapeutic value of the resin than any other so far proposed.

Based upon the average results obtained in the analysis of the several specimens of resin of podophyllum a table was prepared showing moisture, alcohol-insoluble, total resins, the sum of these three factors and "undetermined", the latter being ascertained by subtraction of the sum of the three determined factors from 100 per cent. The values are given in Table 4.

¹ Loc. cit.

TABLE 4.
Summary of the most important findings.

| SPECIMEN | LOSS AT 65°C. | ALCOHOL- INSOLUBLE | RESINS | TOTAL | UNDETER- MINED |
|-----------------|------------------|-----------------------|-----------------|-----------------|-------------------|
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| A | 3 38 | 1.14 | 89 32 | 93 84 | 6 16 |
| B | 3.01 | 1 55 | 88 92 | 93.48 | 6 52 |
| C | 2 34 | 0 48 | 95.06 | 97 87 | 2 13 |
| D | 2 80 | 0 77 | 96 0 | 99 57 | 0 43 |
| E | 2 50 | 0 22 | 92.90 | 95.62 | 4 38 |
| F | 3 38 | 1.26 | 93.48 | 98 12 | 1 88 |
| G | 2 84 | 0 24 | 94.90 | 97 98 | 2 92 |
| H | 1 78 | 1.88 | 86.12 | 89 78 | 10 22 |
| I | 1 73 | 1.08 | 95 38 | 98.19 | 1 81 |
| J Laboratory | 2 94 | 0 21 | 93 75 | 96 00 | 3 10 |

It was observed in general that the specimens showing the greater amounts of "undetermined" were the darker in color, gave the darker solutions in alcohol and contained the smaller amounts of total resins.

In order to obtain further information concerning the practicability of the modified Jenkins method it was sent to several pharmaceutical manufacturers with the request that it be tried on their own products and to several schools of pharmacy with specimens for collaborative study. Only one manufacturer reported. He assayed a part of the same lot (Sample C) which he previously had sent to the Drug Research Unit. His findings averaged 96.5 per cent of resin as compared with an average of 95.3 per cent obtained in the unit. He believed that the method was satisfactory and that it should be included in the next revision of the Pharmacopeia. Only one collaborator from a school of pharmacy reported in time for his results to be included in this paper. His findings for two samples are included in Table 5. The findings obtained in the Drug Research Unit are included for comparison. The data obtained are insufficient to warrant conclusions, but they confirm the findings of the writer.

SUMMARY.

Ten specimens of resin of podophyllum were examined for loss over sulfuric acid, loss on drying at 65°C., ash, proportion soluble in ether, proportion soluble in chloroform, alcohol-insoluble residue, podophyllotoxin and total resins. Determinations of the proportions respectively soluble in ether and in chloroform are of little value. The U. S. P.

TABLE 5.

| SPECIMEN | ANALYST | LOSS AT 65°C. | ASH | TOTAL RESINS | |
|----------|-----------|-----------------|-----------------|-----------------|--|
| D | L. W. D.* | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | |
| | | 3.82 | 1.28 | 94.77 | |
| | | 3.87 | 1.30 | 95.77 | |
| | D. R. U.† | 2.74 | 1.16 | 95.2 | |
| | | 2.86 | 1.08 | 96.3 | |
| | | | | 96.4 | |
| | | | 96.1 | | |
| G | L. W. D.* | 3.67 | 0.55 | 95.02 | |
| | | 3.59 | 0.53 | 95.60 | |
| | D. R. U.† | 2.87 | 0.32 | 94.90 | |
| | | 2.80 | 0.28 | 94.95 | |
| | | | | 95.15 | |
| | | | | | |

* L. W. Daniel, School of Pharmacy, Medical College of Virginia.

† Drug Research Unit.

method for determining these factors is unsatisfactory, and a better method is suggested. Determinations of moisture and alcohol-insoluble material are helpful. The results for podophyllotoxin are consistent, and they possess comparative value, but this determination is not recommended as an assay because the resin contains other active ingredients besides podophyllotoxin. An adaptation of the Jenkins process for the assay of podophyllum was used for determining total resins. The method is the most satisfactory of any process tried. It is believed that a satisfactory evaluation of resin of podophyllum may be obtained by determining the moisture, ash, alcohol-insoluble fraction and the total resins by the modified Jenkins method as proposed.

The writer wishes to acknowledge his appreciation to the several pharmaceutical manufacturers and to others connected with schools of pharmacy who have assisted in this study, either by contributing material or by collaborative work. His thanks are also due the staff of the Pharmacological Laboratory of the Food, Drug and Insecticide Administration for ascertaining the physiological activity of the several fractions of resin of podophyllum on cats, and to William R. Carter for carrying out a number of the routine determinations and for checking many of the other analyses.

IRON, COPPER AND MANGANESE CONTENT OF SOME COMMON VEGETABLE FOODS.

By ROE E. REMINGTON and H. E. SHIVER (Chemical Laboratory of the
South Carolina Food Research Commission, Charleston¹).

Most convincing evidence of the value of traces of copper as an adjunct to iron in hemoglobin building in the rat has been presented by workers from the University of Wisconsin (Waddell, Elvehjem, Steenbock and Hart²). Other investigations of McHargue³, of Titus and Hughes⁴, and of Bertrand and Nakamura⁵ seem to show that manganese may also supplement iron in this respect. While the practical bearing of these observations on normal human and animal nutrition and on the clinical treatment of the various anemias is still some distance in the future, information as to the quantities of these elements contained in various substances which are used as food is desirable. Peterson and Lindow⁶ have drawn attention to the fact that while knowledge of the carbohydrate, fat, and protein content of foodstuffs grown under different conditions and in different localities is very extensive, no such literature exists regarding mineral elements. These authors, together with Elvehjem⁷, have performed valuable service in determining the iron, manganese, and copper content of a wide variety of vegetable foods. While their results are reported in practically all cases on the analysis of a single sample of each kind they have also called attention to possible variations in any given crop. Previous work in this laboratory by Remington, Culp and von Kolnitz⁸ has shown that large variations exist in the iodine content of vegetable foods, while Greaves and Hirst⁹ have called attention to similar variations in the quantities of sodium, potassium, calcium, magnesium, sulfur, phosphorus, and iron present in cereal grains. For purposes of practical value in nutrition work it is desirable that limits of variation and average values be determined.

In connection with the studies which have been made in this laboratory on the iodine content of foods, a large number of samples of vegetables grown in South Carolina were obtained through the cooperation of the several county agricultural and home demonstration agents of the state. Eighteen of the more commonly used vegetables were selected for the study. While it might be desirable to examine a larger number of samples of the same kind, it is believed that when five or more are shown the averages are fairly reliable for this general locality.

The samples were dried by a current of air at room temperature, then

¹ With the technical assistance of C. B. Anderson and Henry McCormack.

² *J. Biol. Chem.*, 77, 797 (1928).

³ *Am. J. Physiol.*, 77, 245 (1926).

⁴ *J. Biol. Chem.*, 80, 565 (1928).

⁵ *Compt. rend.*, 186, 1480 (1928).

⁶ *Soil Sci.*, 26, 149 (1928).

⁷ *J. Biol. Chem.*, 78, 215 (1928); 82, 465 (1929); 75, 169 (1927).

⁸ *J. Am. Chem. Soc.*, 51, 2942 (1929).

⁹ *J. Nutrition*, 1, 293 (1929).

TABLE 1.
Iron, manganese, and copper content of some common vegetables.

| VARIETY | IRON (PARTS PER MILLION, DRY BASIS) | | | | MANGANESE (PARTS PER MILLION, DRY BASIS) | | | | COPPER (PARTS PER MILLION, DRY BASIS) | | | |
|------------------|-------------------------------------|--------------|---------|-------------------|--|--------------|---------|-------------------|---------------------------------------|--------------|---------|-------------------|
| | Maxi- mum | Mini- mum | Average | No. of Samples | Maxi- mum | Mini- mum | Average | No. of Samples | Maxi- mum | Mini- mum | Average | No. of Samples |
| | | | | | | | | | | | | |
| Asparagus ... | 11.0 | 5.3 | 6.6 | 16 | 340 | 125 | 207 | 8 | 86.0 | 27.7 | 42.1 | 9 |
| Bets | 12.0 | 8.7 | 9.9 | 4 | 240 | 152 | 141 | 6 | 85.1 | 33.1 | 55.4 | 6 |
| Beet leaves ... | 14.1 | 7.2 | 9.6 | 3 | 555 | 170 | 372 | 3 | 205.2 | 143.0 | 182.7 | 3 |
| Beans, string... | 10.1 | 5.7 | 8.5 | 15 | 370 | 140 | 201 | 13 | 55.9 | 16.2 | 35.2 | 14 |
| Cabbage..... | 10.6 | 6.2 | 7.7 | 8 | 305 | 74 | 132 | 7 | 47.7 | 30.7 | 40.7 | 8 |
| Carrots | 10.8 | 8.5 | 10.0 | 8 | 330 | 111 | 204 | 8 | 90.9 | 19.1 | 42.2 | 8 |
| Carrot leaves... | 23.0 | 13.6 | 17.3 | 5 | 765 | 355 | 517 | 5 | 199.1 | 51.6 | 121.1 | 5 |
| Egg-plant..... | 7.5 | 5.7 | 6.6 | ... | ... | ... | ... | 3 | 39.2 | 19.7 | 31.1 | 3 |
| Lettuce..... | 10.5 | 4.9 | 6.5 | 9 | 4830 | 425 | 2110 | 8 | 165.2 | 68.2 | 118.4 | 6 |
| Okra..... | 16.7 | 9.9 | 12.9 | 5 | 140 | 72 | 101 | 5 | 62.5 | 36.5 | 48.3 | 8 |
| Onions..... | 15.3 | 6.3 | 11.2 | 6 | 265 | 75 | 156 | 5 | 96.2 | 47.7 | 63.3 | 9 |
| Potatoes..... | 22.7 | 16.8 | 19.6 | 19 | 185 | 65 | 99 | 39 | 14.3 | 5.6 | 9.4 | 43 |
| Spinach..... | 12.9 | 5.4 | 9.1 | 13 | 1750 | 275 | 956 | 12 | 253.3 | 52.5 | 141.2 | 14 |
| Squash..... | 6.6 | 5.4 | 6.0 | 3 | 130 | 70 | 105 | 6 | 27.6 | 18.9 | 23.8 | 6 |
| Sweet potatoes. | 33.7 | 27.3 | 29.9 | 11 | 88 | 39 | 64 | 9 | 17.7 | 2.6 | 9.3 | 10 |
| Turnips..... | 10.9 | 8.0 | 9.2 | ... | ... | ... | ... | 5 | 19.3 | 9.7 | 13.6 | 5 |
| Turnip leaves.. | 16.8 | 11.4 | 13.2 | ... | ... | ... | ... | 6 | 143.8 | 64.0 | 107.6 | 5 |
| Tomatoes..... | 8.5 | 3.0 | 4.7 | 8 | 185 | 115 | 148 | 8 | 33.7 | 17.7 | 25.7 | 8 |

in an oven at 80°C., after which they were ground and bottled. Although results are reported as on the dry basis, the ground samples still contained from 1 to 3 per cent of moisture as determined by drying the powder at 100°C. For the determination of copper and manganese, respectively, 5 grams of dried vegetables were used, and 1 gram was used for the determination of iron. The methods followed, those of the Wisconsin workers, were subjected to a preliminary series of careful recovery experiments on known amounts of the metals sought and found to be as reliable as the authors reported them to be. For purposes of comparison with the roots, the leaves of beets, carrots, and turnips were included. Carrot leaves may not be eaten, but beet leaves frequently are and turnip leaves are probably more popular than any other salad green in this locality. The results are summarized in Table 1.

It is apparent from the results given that among the several vegetable types there is a far wider degree of variation in iron and manganese than in copper. In iron the range is from an average of 2110 parts per million in lettuce to 64 in sweet potatoes, for manganese from 183 in beet leaves to 9.3 in sweet potatoes, while for copper it is only from 17.4 in tomatoes to 4.4 in turnips. The variation among samples of the same product is also much wider in iron and manganese than in copper, and greater in iron than in manganese. The significance of these wide variations is not as yet apparent. Greaves and Hirst were able to find a correlation between the quantity of available soil moisture and the mineral content of grain, but it seems extremely doubtful that available moisture can be the predominant factor in these South Carolina vegetables.

In comparison with the values reported by the Wisconsin workers, the average values for iron and manganese reported in this paper are on the whole higher, in some cases very much higher. Out of thirteen varieties on which comparative values were obtained, ten South Carolina samples gave higher values for iron, while all of eleven varieties which could be compared showed higher values for manganese. On copper the differences are not so striking, all of the Wisconsin values falling within the range of the samples here reported. Of fifteen varieties which could be compared, averages on six are higher. The high concentration of copper in tomatoes is of interest in connection with striking effects that have been produced in Florida by Allison, Bryan and Hunter¹ by fertilizing this crop with a small quantity of copper sulfate.

If these vegetables are divided into groups, some interesting relations develop. Iron, manganese, and copper in leaves and shoots average, respectively, 682, 108, and 11 parts per million; roots and tubers, in the same constituents average 133, 32.2 and 8.2 parts per million; likewise, fruits average 139, 32.8 and 12.5 parts per million, all on a dry basis.

¹ Florida Agri. Expt. Sta. Bull., 190 (1927).

Leafy vegetables were found to be outstanding in their content of iron and manganese, and while the differences with regard to copper are not so striking, the results on roots are definitely lower than those on leaves and fruits. This observation confirms that of the Wisconsin workers, and helps to establish the predominant value of the green leafy vegetables in the diet. It also parallels previous observations from this laboratory on the iodine content of vegetables.

Three root crops—beets, carrots, and turnips—were examined both as to roots and foliage. The comparative values are of interest and are shown in Table 2.

TABLE 2.

Iron, manganese, and copper content of roots and leaves of the same plant compared

(Parts per million.)

| | IRON | MANGANESE | COPPER |
|-------------------------|------|-----------|--------|
| Beet roots | 141 | 55.4 | 9.1 |
| Beet leaves | 372 | 182.7 | 13.5 |
| Carrot roots | 204 | 42.2 | 10.7 |
| Carrot leaves | 517 | 121.1 | 12.4 |
| Turnip roots | ... | 13.6 | 4.4 |
| Turnip leaves | ... | 107.6 | 7.8 |

The differences shown in the results in Table 2 cannot be due to differences in water content, for if calculated back to the original basis they are even more striking. It is customary to think of the foliage as the active part of the plant in metabolism, the root in such crops as these being principally storage tissue, and these differences may perhaps be indirect evidence of the essential function of these metals in the metabolism of the plant.

SUMMARY.

Data are presented for the iron, manganese, and copper content of a number of samples of eighteen kinds of vegetables which are used as food. The methods of analysis are those employed by workers at the University of Wisconsin. They were checked on known quantities of the element sought and found satisfactory. The results are discussed in the light of previously published data and from the standpoint of the value of these vegetables in nutrition. When compared with root and fruit vegetables, the green leafy vegetables were found to be outstanding as sources of iron, manganese, and copper.

CITRATE-SOLUBLE PHOSPHORIC ACID IN COLLOIDAL PHOSPHATE.

By J. B. MARTIN and EDMUND C. SHOREY (Bureau of Chemistry and Soils, Washington, D. C.).

In the course of the analytical control of fertilizers used in the experimental work of the Division of Soil Fertility of the Bureau of Chemistry and Soils, there have been encountered, from time to time, materials containing phosphates in a colloidal condition. When such phosphates or mixed fertilizers containing them are analyzed by the official methods of the A. O. A. C. to determine the citrate-insoluble phosphoric acid, it frequently happens that a cloudy filtrate is obtained in the water and in the ammonium citrate extracts. Since the available phosphoric acid is usually regarded as the water-soluble plus that portion of the water-insoluble that is soluble in ammonium citrate, it follows that if any of the insoluble phosphate runs through the filter after the digestion with ammonium citrate and causes a cloudiness in the filtrate, the available phosphoric acid found by this method will be higher than that actually present.

That the colloidal phosphate that is insoluble in ammonium citrate causes this turbidity is easily shown. Two samples of untreated phosphates in a very finely divided condition, one a rock phosphate and the other a so-called soft phosphate, were examined. One gram of material was used in each case. The results are as follows:

| | WATER EXTRACT | AMMONIUM CITRATE EXTRACT |
|---------------------|---------------|-----------------------------|
| Rock Phosphate... . | Clear | Cloudy |
| Soft Phosphate... . | Cloudy | Very Cloudy |

The ammonium citrate extract of the rock phosphate was filtered several times through another filter until a clear filtrate was obtained. The phosphoric acid in the precipitate was found to be 0.23 per cent of the original sample, which contained 30.77 per cent of phosphoric acid.

Continued refiltering of the water and ammonium citrate extracts from the soft phosphate failed to give a clear filtrate, but the addition of sodium chloride until a 10 per cent solution was formed flocculated the colloidal material so that it filtered readily, a clear filtrate being obtained. The phosphoric acid in the residue so obtained was 0.30 per cent in the case of the water extract and 2.83 per cent in the case of the citrate extract, both figures being a percentage of the original material. The total phosphoric acid in this material was 25.19 per cent and the citrate-insoluble, determined by the official method, disregarding the turbidity of the filtrate, was 19.17 per cent.

It was found that a 2 per cent solution of ammonium chloride was as effective in flocculating the colloidal phosphate as a 10 per cent solution

of sodium chloride; accordingly, this same sample was washed with 2 per cent ammonium chloride, then digested with ammonium citrate as in the official method, and washed with 2 per cent ammonium chloride. In this way 22.34 per cent citrate insoluble was obtained, which is in good agreement with the sum of the quantities obtained by the separate treatments, viz. 19.17 per cent insoluble by the official method, 0.30 per cent obtained from the turbid water extract, and 2.83 per cent obtained from the turbid ammonium citrate extract, a total of 22.30 per cent.

The official method for the determination of citrate-soluble phosphoric acid in non-acidulated material does not provide for the preliminary procedure outlined above. Accordingly, the same soft phosphate was treated as follows: Two grams was digested with 100 cc. of ammonium citrate in the regular way, and after digestion and before filtration, 2 grams of ammonium chloride was added, filtered and washed with a 2 per cent solution of ammonium chloride.

The filtrate and washings were clear, and the citrate insoluble was found to be 22.35 per cent and 22.37 per cent in duplicate determinations. The average, 22.36 per cent, is in good agreement with the figures previously obtained, viz. 22.30 and 22.34 per cent.

To determine the effect that might be brought about through using 10 per cent sodium chloride solution for washing in place of water in the case of a complete fertilizer, such a fertilizer made up 12-24-12 was analyzed for phosphoric acid in the regular way, a water extract being made first and then water used for washing after ammonium citrate; and second, 10 per cent sodium chloride was used in place of water. Both the filtrates were clear when sodium chloride was used, and when water was used there was only a slight trace of turbidity. When sodium chloride was used, the water-soluble phosphoric acid was 24.10 per cent; and when water only was used, it was 24.09 per cent. The citrate insoluble was found to be the same in both cases—0.68 per cent.

As noted above, a 2 per cent ammonium chloride solution was found as effective in flocculating as 10 per cent sodium chloride, and this was also true of 2 per cent ammonium nitrate; and further, it was found that the sodium chloride solution could be reduced to 5 per cent.

When the after treatment of the insoluble residue is considered, 2 per cent ammonium chloride seems to be the preferable salt to use, especially if the ignition with magnesium nitrate is resorted to, since the salt is completely volatilized and does not fuse and leave a mass difficult to remove from the dish as is the case with sodium chloride.

The filter papers used in this work were Whatman No. 44, C. C. & S. No. 589, Blue Ribbon and Munktell No. 00, and while there was some difference in time of filtration with different papers, there was not much difference in the degree of turbidity of filtrates. It should be noted

that when sodium chloride or other salt was used for flocculation the filtration was about four times as fast as with water alone.

The official method for water-soluble phosphoric acid provides for the addition of 1-2 cc. of strong nitric acid if the solution is turbid. In the case of the turbid solutions discussed in this paper such addition of nitric acid did not clear them, although in some cases it made them less turbid. The turbidity referred to in the official method is that which sometimes appears in the water extract of acidulated phosphates on standing. A turbidity from colloidal insoluble phosphate would not be cleared up by dilute nitric acid.

The sum of the water-soluble phosphoric acid and the citrate-soluble portion of the water-insoluble phosphoric acid is frequently spoken of and regarded as available phosphoric acid; and in this connection it is sometimes suggested or assumed that the colloidal phosphate is also available. The assumption that the water-soluble plus the citrate-soluble phosphoric acid is available is based on extensive experimental field work, but in the case of the colloidal phosphate the volume of work is not so great nor so conclusive.

The following points may be made in this connection:

(1) One of the objects of filtration in analytical operations is to obtain a clear filtrate; and when this result is not obtained it is obvious that the particular method used is not applicable to the material being analyzed.

(2) When the official method is used for the determination of citrate-soluble phosphoric acid in certain phosphatic materials a turbid filtrate is obtained, and it has been shown that the turbidity is due partly to colloidal phosphate that ordinary filter papers will not retain.

(3) It follows, then, that the official method is not applicable to such material, and further, that if this turbidity is ignored the citrate-soluble phosphoric acid reported will be too high—as high as 2 or 3 per cent on material containing a total of 20-25 per cent.

(4) Several available methods prevent this turbidity in the filtrate from ammonium citrate digestion and apparently give a true figure for citrate-soluble phosphoric acid, but obviously such methods are not official.

(5) If available phosphoric acid is interpreted to mean water-soluble plus citrate-soluble phosphoric acid, the results reported when the official method is used may be too high in material containing colloidal phosphate.

(6) If, on the other hand, it is assumed that colloidal phosphate has an availability equal to that of citrate soluble, the figures obtained by the official method may be too low, since it cannot be assumed that all the colloidal available phosphate will pass through the filter paper.

No originality is suggested here for the use of sodium chloride in flocculating the colloidal phosphate. It was mentioned in discussion¹ at the last meeting of the A. O. A. C. and, in any case, the use of some electrolyte for this purpose might be regarded as an "obvious expedient".

It is suggested that when either the water or ammonium citrate extracts cannot be filtered clear, owing to the presence of colloidal phosphate, a 2 per cent solution of ammonium chloride be substituted for water in the determination of water-soluble phosphoric acid, and in the case of citrate-insoluble phosphoric acid, that ammonium chloride to make a 2 per cent solution be added to the ammonium citrate digestion before filtration and the residue be washed with a 2 per cent solution of ammonium chloride.

SOME OBSERVATIONS ON THE APPLICATION OF THE FORMOL TITRATION TO HONEY².

By H. A. SCHUETTE and VERA TEMPLIN (Laboratory of Foods and Sanitation, Department of Chemistry, University of Wisconsin, Madison, Wis.).

The extensive studies by Schiff³ of the mechanism of the interaction of neutralized formaldehyde and ammonium salts and of the formation of methylene substitution products through the agency of this aldehyde afforded a new means of attack upon the study of proteins. With the results of these investigations as a background, Sørensen⁴ perfected the so-called "formol-titrations", by virtue of which the quantitative estimation of the acidic groups in amino acids and proteins is made possible.

Numerous practical applications of this reaction were made in the field of biological chemistry after its sponsor had utilized it for the measurement of the velocity of hydrolysis of proteins by enzymes. Pertinent among these applications will be found one by Tillmans and Kiesgen⁵, who utilized this procedure for the analysis of lemon juice, vinegar, and honey and more recently that by Hill⁶, who adapted it to the differentiation of natural from artificial citrus juices, vinegar, etc.

In view of the above recommendation given the formol titration as a means of detecting the admixture of foreign sirups with honey, investigations under way in this laboratory on this food product were made to include a critical study of the practicability of the Tillmans-Kiesgen procedure⁵ when applied to American-grown honeys. The presentation

¹ Annual Report of Florida State Chemist, 38: 24-6 (1928).

² Presented before the Division of Agricultural and Food Chemistry at the 77th Meeting of the American Chemical Society, Columbus, Ohio, April 29 to May 3, 1929, and published here by courtesy of *Industrial and Engineering Chemistry*.

³ *Ann.*, 310, 25 (1899); 319, 59, 287 (1901); 325, 348 (1902).

⁴ *Compt. rend. trav. lab. Carlsberg*, 7, 1 (1907); *Biochem. Z.*, 7, 45 (1908).

⁵ *Z. Nahr. Genussm.*, 53, 181 (1927).

⁶ *Chem. Eng. Mining Rev.*, 20, 401 (1928); *C. A.*, 23, 1180 (1929).

of the data obtained in this phase of the study is the object of this communication. The observations pertinent to certain difficulties which are encountered in the performance of this titration are also presented, as well as a comparison of the nitrogen content of honey as determined, on the one hand by the formol titration and, on the other, by the familiar Kjeldahl procedure¹.

DESCRIPTION OF METHOD.

Experimental material.—Fifteen domestic honeys, which had been grown in widely separated regions, were used for experimental study. All were laevo-rotatory; three, though showing an ash content slightly higher than the permitted maximum for a legal honey (0.25 per cent), yet were normal with respect to moisture and sucrose requirements; one had evidently been removed from the hive before it was fully "ripe"; and one was unmistakably acid. With the exception of two commercial samples, which were apparently blended products, all were the output of individual apiaries.

Method.—A 40 gram sample of honey was dissolved in about 100 cc. of water, treated with 0.2 cc. of a 2 per cent solution of phenolphthalein, and then neutralized with 0.1 *N* sodium hydroxide solution to a pink end point. The whole mixture was then diluted to 200 cc., thoroughly mixed, and divided into two parts, Nessler tubes serving the purpose very well. To the contents of one tube 10 cc. of neutralized formaldehyde (40%) was added, to the other 10 cc. of boiled distilled water; 0.05 *N* sodium hydroxide solution was then added to the first tube until the color again matched that of the second. The volume of sodium hydroxide solution required to restore the original alkaline condition, when expressed as cubic centimeters of 0.1 *N* sodium hydroxide, is the "formol titration" of 20 grams of honey.

OBSERVATIONS ON THE FORMOL TITRATION.

The average formol titration (Table 1) of the experimental honeys was found to be 0.41 cc., a value approximately one-half of that reported by Tillmans and Kiesgen as being characteristic of normal honeys of German origin. Minimum and maximum values were 0.25 and 0.76 cc., respectively. Only two of the values exceeded unity. Since this appears to be an abnormal condition due to soured samples, the pertinent data were not included in the averages.

With the formol titration values falling within a comparatively narrow range and rising to a maximum whose order of magnitude does not appear to be striking, it is doubtful, in the opinion of the writers, if the

¹ *Methods of Analysis*, A. O. A. C., 1925, p. 8.

method of procedure as at present constituted¹ possesses sufficient promise of development to become a useful tool *per se* for detecting the admixture of foreign sirups with honey. Especially is this true when certain factors in the procedure play such a large part in the determination of this value. The concentration of the indicator used in the neutralization of the honey and of the formaldehyde exerts a material effect upon the final results. Difficulties are frequently met with in the neutralization of the formaldehyde, although they might perhaps be reduced by the procedure suggested by Shaw². If the amount of alkali required to complete the neutralization after the addition of the formaldehyde is in the neighborhood of 0.5 cc., any variation in the excess of alkali in the formaldehyde itself makes considerable difference. The fact that the end point of the neutralization of the free acidity of the honey shows a decided tendency to fade makes the starting point of the formol titration rather variable. Satisfactory duplication of results is difficult for these reasons.

TABLE 1.

Formol titration of honey.

| FLORAL NECTAR | SOURCE | 0.1 N NaOH PER 20 GRAM SAMPLE |
|--------------------|------------------|----------------------------------|
| | | cc. |
| Clover..... | Wisconsin | 0.25 |
| "..... | " | 0.33 |
| "..... | " | 0.50 |
| "..... | " | 0.52 |
| "..... | Kansas | 0.38 |
| Orange blossom... | California | 0.26 |
| Spanish needle.... | Missouri | 1.33* |
| Unknown.. . . | Alabama | 0.53 |
| "..... | " | 0.60 |
| "..... | Wisconsin | 1.03* |
| "..... | " | 0.47 |
| "..... | " | 0.50 |
| "..... | " | 0.53 |
| Blended product.. | Ohio | 0.53 |
| "..... | New York | 0.76 |
| Average | of normal honeys | 0.41 |

* Sour honey.

¹ Some time after this study was terminated there appeared a communication by Gottfried [*Z. Unters. Lebensm.*, 57, 558 (1929)] in which the author revealed that he found the formol titration values of nine honeys in which adulteration was alleged to lie between 0.3 and 1.2 cc. Examination of some eighty pure honeys disclosed a range of formol titration values of which only 12.5 per cent fell below 1 cc. Exactly 15 per cent of this group showed a value of 1 cc., over 50 per cent were in the 1 to 2 cc. bracket, whereas some 17.5 per cent fell in the 2 to 4 cc. group. In the light of these data, Gottfried is of the opinion that the formol titration is a valuable aid in differentiating between genuine and sophisticated honeys.

² *Analyst*, 49, 558 (1924).

EFFECT OF ADDED SUGAR SIRUPS.

In order to establish a definite picture of the effect which is produced upon the formol titration of genuine honeys when foreign sirups are added, there were added to six authentic honeys varying proportions, respectively, of a so-called "crystal white" commercial glucose and confectioners' invert sirup. These synthetic mixtures were then subjected to analysis as described in preceding paragraphs. Both adulterants gave negative formol titrations.

TABLE 2.
Formol titration values of honey and sirup mixtures.

| HONEY | CONFECTIONERS' INVERT SIRUP PER CENT ADDED | | | | COMMERCIAL GLUCOSE PER CENT ADDED | | | |
|-------------------------|---|------|------|------|--------------------------------------|------|------|------|
| | 0 | 12.5 | 25 | 50 | 0 | 12.5 | 25 | 50 |
| FORMOL TITRATION VALUES | | | | | | | | |
| Clover | 0.38 | 0.30 | 0.13 | 0.00 | | | | |
| " | 0.50 | 0.33 | 0.26 | 0.00 | | | | |
| Spanish needle . . | 1.00 | 0.93 | 0.73 | 0.47 | 1.00 | 0.98 | 0.90 | 0.67 |
| Unknown | 0.47 | 0.47 | 0.27 | 0.23 | 0.47 | 0.43 | 0.43 | 0.39 |
| " | 0.50 | 0.50 | 0.43 | 0.43 | 0.50 | 0.46 | 0.40 | 0.26 |
| " | 0.53 | 0.53 | 0.47 | 0.30 | 0.53 | 0.52 | 0.41 | 0.37 |

Figure I, illustrating the data of Table 2, shows in a graphic manner how the initial formol titration values of the experimental honeys were depressed by the addition of an adulterant. That the expected arithmetical decrease was not obtained is due not only to those idiosyncrasies of this titration which have been commented on in preceding paragraphs, but also to the narrow margin of operation within which it appears that it must be conducted when applied to honey. Under such circumstances the error incident to the personal equation is multiplied.

CALCULATION OF THE NITROGEN CONTENT.

It should be possible to calculate the nitrogen content from the formol titration value if one may assume that for each carboxyl there is present an equivalent amino group. The average calculated nitrogen content (Table 3) of twelve honeys, however, proved to be 0.004 per cent in contrast to the value 0.036 obtained experimentally by the Kjeldahl procedure. The formol titration on this basis indicated only 11 per cent of the total nitrogen content.

Inasmuch as the probable source of nitrogen in a honey is the pollen inadvertently or otherwise introduced by the bee, it would appear that the abnormalities indicated in this case might very well be traced to the

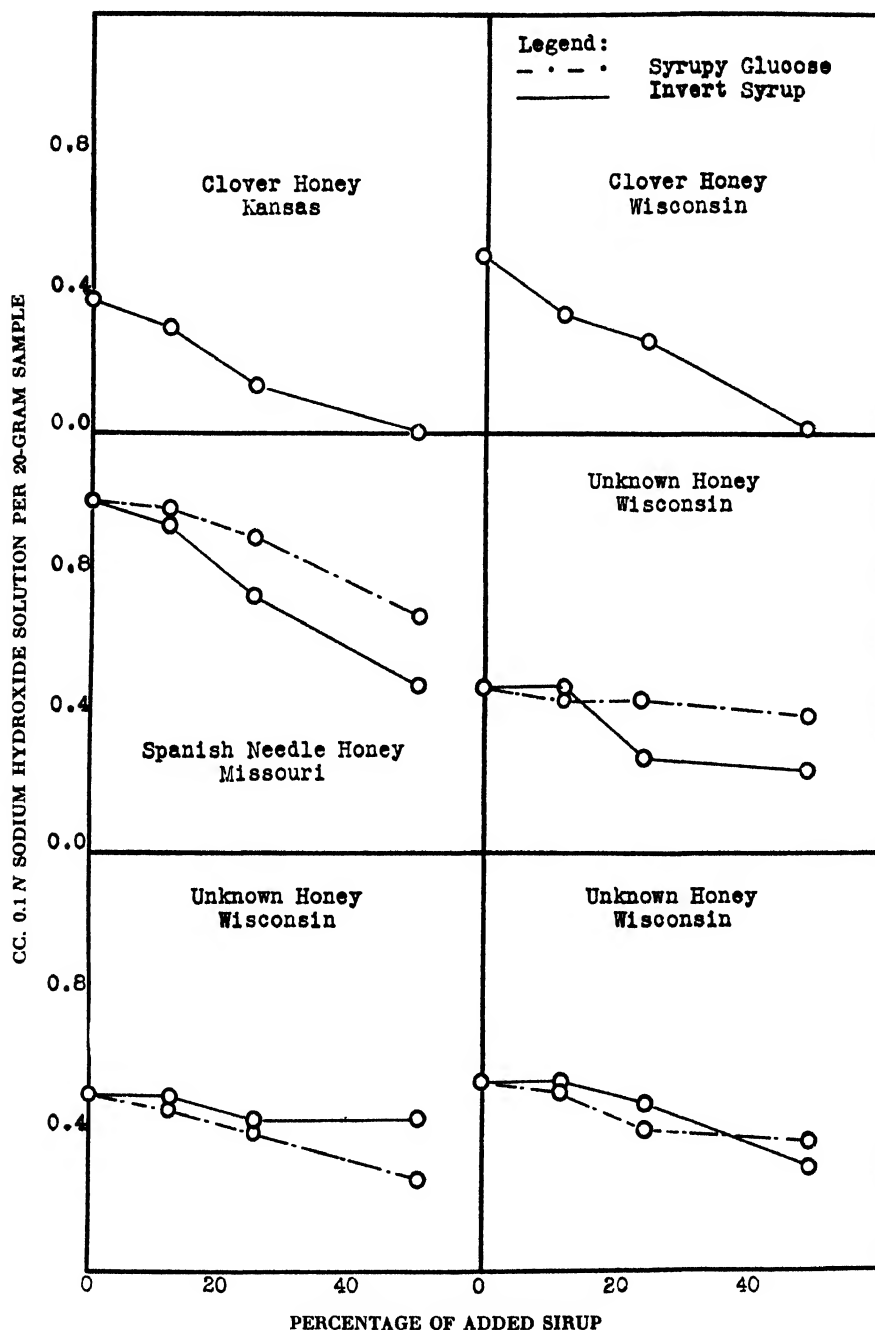


FIGURE I.—FORMOL TITRATION VALUES OF HONEY-SIRUP MIXTURES.

TABLE 3.
Nitrogen content of honey.

| FLORAL NECTAR | NITROGEN CONTENT | | TOTAL NITROGEN INDICATED BY FORMOL TITRATION |
|----------------------|------------------|-----------------|--|
| | Determined | Calculated | |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Clover | 0.017 | 0.0017 | 10.0 |
| " | 0.024 | 0.0035 | 14.6 |
| " | 0.025 | 0.0023 | 9.2 |
| " | 0.031 | 0.0027 | 8.7 |
| " | 0.037 | 0.0036 | 9.7 |
| Orange blossom..... | 0.018 | 0.0018 | 10.0 |
| Spanish needle..... | 0.053 | 0.0093 | 17.5 |
| Unknown..... | 0.027 | 0.0037 | 13.7 |
| " | 0.038 | 0.0041 | 10.8 |
| " | 0.069 | 0.0072 | 10.4 |
| Blended product..... | 0.025 | 0.0037 | 14.8 |
| " | 0.048 | 0.0053 | 11.0 |
| Average | 0.036 | 0.0040 | 11.0 |

behavior of its proteins with formaldehyde. Jodidi¹ advanced the view that the formol titration admits only of accurate results in the case of amino acids which contain in the molecule amino and carboxyl groups alone; that low results may be expected when an amino acid contains also an imino group; and that the results may vary in either direction if a phenol or a guanidine residue constitutes part of the amino acid molecule.

Correlating this view-point are certain facts gleaned from the investigations of Heyl and Hopkins² on the proteins of ragweed pollen. These investigators reported the presence therein of arginine, histidine, tyrosine, and lysine. While, to be sure, it cannot be stated with certainty that these same amino acids will be found in all pollens, yet, because of a common biological origin, there is probably a reasonable basis for the assumption that at least some of them are present in most pollens, and that their presence in the honeys under investigation was a contributing cause of the discrepancies which were noted between the determined and the calculated nitrogen content. Of this group lysine alone yields quantitative results in a formol titration; histidine, containing an imino group, should depress the nitrogen percentage; while arginine, a guanidine derivative, and tyrosine, the α -amino acid of *p*-hydroxyphenylpropionic acid, both give erroneous results.

SUMMARY.

The average formol titration value of fifteen normal honeys was found to be 0.4 cc. 0.1 *N* alkali for a 20 gram sample, the minimum

¹ *J. Am. Chem. Soc.*, **40**, 1031 (1918).

² *Ibid.*, **42**, 1738 (1920).

and maximum being 0.25 cc. and 0.76 cc., respectively. This value is approximately one-half of that reported by Tillmans and Kiesgen as being characteristic of honeys of German origin. It has been observed that those factors which make duplication of results difficult center around the concentration of the solutions, the concentration of the indicator, and the small but unavoidable excess of alkali in the neutralized formaldehyde.

Although it is true that the admixture of foreign sugars of the type represented by commercial invert sugar sirup or sirupy glucose causes a fairly consistent though slight decrease of the formol titration value with increased addition of adulterant, yet the very low order of magnitude of the average initial value does not recommend the procedure of itself alone as a means of establishing the genuineness of a honey whose purity may be in dispute. Its value might perhaps lie in the suggestion that when corroborated by supporting analytical data it may perhaps have a limited diagnostic value.

In the light of the evidence herein reported, it may be said that the formol titration value does not afford an accurate measure of the protein content of honey.

EFFECT OF METHOD OF RENDERING ON THE REFRACTIVE INDEX OF FATS*.

By HARRY W. BLOCK (Purdue University Agricultural Experiment Station).

In the routine analysis of fats the determination of refractive index is often an important test for identification. It is also important in the study of the type of fat developed by animals on limited rations when it is used as a measure of the "firmness" of the fat. Ellis and co-workers^{1 2 3 4} found that as hogs mature the fat becomes harder and the refractive index of the fat decreases. They also found that the ration fed the animals affected the fat deposited and hence also the refractive index of the fat. Anderson and Mendel⁵, working with albino rats, observed a decrease in refractive index with increase in weight of the animals. Eckstein⁶ considered that this lowering of refractive index is more dependent on the diet than on the weight or maturity of the animal. Hankins and Ellis^{3 4} attempted to formulate a table correlating the refractive index with the firmness of the fat. They later modified

*The material presented is a part of the dissertation submitted for the degree of master of science at Purdue University. Appreciation is extended to Dr. H. R. Kraybill for his many helpful suggestions.

¹ *J. Biol. Chem.*, 66, 101 (1925).

² *Ibid.*, 69, 239 (1926).

³ U. S. Dept. Agr. Bull. 1407 (1926).

⁴ *Ibid.*, 1492 (1928).

⁵ *J. Biol. Chem.*, 76, 729 (1928).

⁶ *Ibid.*, 81, 613 (1929).

this table to correspond to different rations. In any such table the narrow range in refractive index between the different grades makes it difficult to differentiate the grades accurately. Hence any factors influencing the refractive index may influence the grade attributed to the fat.

METHODS OF RENDERING FAT.

Lewkowitsch¹ states that the oxidation produced by heating fat causes an increase in the refractive index. Leathes and Raper² also maintain that heating the fat in an open dish affects the refractive index. In order to prevent this oxidation Frankel³ suggests that the fat tissue should not be heated over 70°C. when it is rendered. He prefers to grind the tissue with some anhydrous salt and to extract the fat with anhydrous petroleum ether. Anderson and Mendel⁴ rendered the fat by autoclaving and later filtered off the melted fat.

Probably the most common method of rendering fat tissue is by heating. However, in the literature it is unusual to find exact descriptions of the methods used for rendering. Most investigators describe their general methods but fail to give exact conditions of treatment. Hence a study was made of the various ways of treating fat tissue in order to note the effect on refractive index.

EXPERIMENTAL WORK.

Four pieces of back fat, obtained from a local packing house, were ground and mixed. Aliquots were placed in one-half inch test tubes (for narrow-mouthed containers) and in 150 cc. beakers (for wide-mouthed containers). One set of samples was heated 6, 8, and 10 hours at 50°, 70°, 100°, and 110°C. in an air oven. A test-tube sample of each was kept in an atmosphere of nitrogen (by passing nitrogen gas into the test tubes) for 8 hours at each of the above temperatures. The refractive indices of these samples are given in Table 1. Other samples of each piece of back fat were mixed with anhydrous sodium sulfate and disodium phosphate and then extracted with anhydrous petroleum ether (b. p. below 60°C.); another set of samples was merely extracted without drying; and two other sets of samples were autoclaved for 8 hours at 15 pounds pressure—one in sealed Pyrex tubes and the other in open tubes.

Since Trowbridge⁵ had suggested drying meat in vacuo at freezing temperature, an attempt was made to observe whether his method could be applied to the drying of adipose tissue. One set of samples was dried

¹ Chemical Technology and Analysis of Oils, Fats and Waxes, 6th ed. (1921), 1, p. 365.

² The Fats (1925), p. 54.

³ Abderhalden Handbuch der Biol. Arbeitsmethoden, abt. I Teil 6, pp. 1-20.

⁴ J. Biol. Chem., 76, 729 (1928).

⁵ U. S. Dept. Agr. Bur. Chem. Bull. 122, p. 219.

TABLE 1.

Comparison of refractive indices at 40°C. of fats rendered by heating in an air oven at different temperatures and for varying periods of time.

| NO. | 6 HOURS | | 8 HOURS | | 8 HOURS | 10 HOURS | |
|------------------|----------------|------------------------------|----------------|------------------------------|----------------------------|----------------|------------------------------|
| | 150 cc. beaker | $\frac{1}{2}$ inch test tube | 150 cc. beaker | $\frac{1}{2}$ inch test tube | Test tube in N. atmosphere | 150 cc. beaker | $\frac{1}{2}$ inch test tube |
| HEATED AT 50°C. | | | | | | | |
| 1 | 1.4592 | 1.4592 | 1.4592 | 1.4592 | 1.4592 | 1.4590 | 1.4592 |
| 2 | 1.4603 | 1.4603 | 1.4604 | 1.4604 | 1.4603 | 1.4604 | 1.4607 |
| 3 | 1.4600 | 1.4599 | 1.4600 | 1.4600 | 1.4598 | 1.4601 | 1.4603 |
| 4 | 1.4592 | 1.4591 | 1.4592 | 1.4592 | 1.4590 | 1.4592 | 1.4595 |
| HEATED AT 70°C. | | | | | | | |
| 1 | 1.4596 | 1.4595 | 1.4595 | 1.4594 | 1.4594 | 1.4596 | 1.4595 |
| 2 | 1.4606 | 1.4604 | 1.4605 | 1.4604 | 1.4604 | 1.4605 | 1.4604 |
| 3 | 1.4599 | 1.4604 | 1.4601 | 1.4603 | 1.4601 | 1.4601 | 1.4601 |
| 4 | 1.4597 | 1.4596 | 1.4597 | 1.4593 | 1.4594 | 1.4596 | 1.4595 |
| HEATED AT 100°C. | | | | | | | |
| 1 | 1.4597 | 1.4592 | 1.4596 | 1.4592 | 1.4593 | 1.4597 | 1.4592 |
| 2 | 1.4606 | 1.4605 | 1.4607 | 1.4604 | 1.4604 | 1.4608 | 1.4603 |
| 3 | 1.4602 | 1.4600 | 1.4603 | 1.4600 | 1.4600 | 1.4604 | 1.4599 |
| 4 | 1.4596 | 1.4590 | 1.4595 | 1.4592 | 1.4592 | 1.4595 | 1.4593 |
| HEATED AT 110°C. | | | | | | | |
| 1 | 1.4598 | 1.4593 | 1.4599 | 1.4595 | 1.4592 | 1.4599 | 1.4597 |
| 2 | 1.4608 | 1.4605 | 1.4609 | 1.4604 | 1.4603 | 1.4609 | 1.4606 |
| 3 | 1.4604 | 1.4601 | 1.4606 | 1.4602 | 1.4600 | 1.4606 | 1.4603 |
| 4 | 1.4597 | 1.4593 | 1.4598 | 1.4595 | 1.4592 | 1.4601 | 1.4597 |

in a vacuum desiccator over sulfuric acid at room temperature at a high vacuum; another set was treated in a similar manner but in a current of air; and a third set was treated in high vacuum but at freezing temperature. When dry, these samples were ground and extracted with petroleum ether, and the ether was driven off by a fan. The samples were then dried overnight in a vacuum oven at room temperature. The refractive indices of the samples rendered by methods other than by heating in an air oven are given in Table 2.

DISCUSSION.

Rendering in a test tube in an atmosphere of nitrogen, by autoclaving in a sealed tube or by drying with either anhydrous sodium sulfate or anhydrous disodium phosphate and extracting with petroleum ether gave fats of about the same refractive indices. It is believed that these methods of extraction had very little if any effect on the refractive index.

The samples rendered in a test tube ($\frac{1}{2}$ inch in diameter) at temperatures not exceeding 100°C. and for a period of time not longer than 8 hours or at 110°C. for a period not longer than 6 hours show little

TABLE 2.

Refractive indices of samples treated by methods other than heat.

| METHOD OF TREATMENT | SAMPLE NUMBER | | | |
|--|---------------|--------|--------|--------|
| | 1 | 2 | 3 | 4 |
| Not dried [Ext. with petroleum ether (b. p. 60°C.)] | 1.4593 | 1.4603 | 1.4599 | 1.4593 |
| Dried with anhydrous Na ₂ SO ₄ | 1.4591 | 1.4603 | 1.4599 | 1.4593 |
| Dried with anhydrous Na ₂ HPO ₄ | 1.4591 | 1.4604 | 1.4600 | 1.4595 |
| Dried in vacuum at freezing temperature | 1.4601 | 1.4610 | 1.4609 | 1.4600 |
| Dried in vacuum at room temperature | 1.4602 | 1.4609 | 1.4609 | 1.4598 |
| Dried in vacuum with current of air at room temperature. | 1.4600 | 1.4609 | 1.4609 | 1.4600 |
| Autoclaved in sealed tube (not extracted) | 1.4592 | 1.4604 | 1.4599 | 1.4592 |
| Autoclaved in open tube (not extracted) | 1.4589 | 1.4602 | 1.4598 | 1.4590 |

change in refractive index. When the samples were heated for 10 hours at 110°C., the refractive index increased significantly, as it did when the samples were rendered by heating in a 150 cc. beaker at 100°C. for only 6 hours. Evidence that this change was caused by oxidation was obtained from the lowering of the iodine numbers and from positive Kries tests¹ in several instances.

The results show that oxidation occurs if the adipose tissue is heated above 70°C. for a period of time in an air oven. This action is hastened if there is a greater surface exposed to the air; it is prevented in an atmosphere of some inert gas. This oxidation may be avoided by drying with some anhydrous salt followed by extraction with anhydrous petroleum ether. Autoclaving in sealed tubes was probably the best method tried; the moisture that condensed in the open test tubes caused an excessively low refractive index. The cells were well broken down, and the fat could easily be pressed and filtered out and obtained free from the effects of oxidation. Two to three weeks were required to dry the samples in vacuo. The increase in refractive index may have been due to some hydrolysis of the fat. By avoiding too high temperatures or too long heating periods and using containers with a narrow cross section ($\frac{1}{2}$ inch test tubes) samples of rendered fat may be obtained without marked change in refractive index by heating in an air oven.

CONCLUSIONS.

1. Heating fat tissue long periods of time or in open dishes results in an increase in refractive index of the fat, which is due to oxidation.
2. This oxidation of fat can be prevented in large part by heating in an atmosphere of nitrogen.
3. Autoclaving the adipose tissue fat 8 hours at 15 pounds' pressure

¹ Naturforschenden Gesellschaft in Basel, 15, 225 (1903-4).

in sealed tubes is preferable to the other methods tried, since the fat can easily be obtained free from the effects of oxidation.

4. When comparing the refractive index results obtained by different workers, the methods used in rendering the fat must be known and taken into consideration.

NOTES.

Procedure for Determining Total Nitrogen by Conversion to Nitric Acid*.

The following changes and condensations of a procedure previously reported¹ for the determination of total nitrogen by conversion to nitric acid have been found to improve the method for routine analyses:

Put weighed portions of the sample containing at least 0.5 mg. of nitrogen (usually 1.0 gram of soil or green plant tissue or 0.1 gram of dry plant tissue) into dry 500 cc. Kjeldahl flasks. (Green plant tissue may be left intact.) Add 1 gram of sodium chlorate for each 0.5 gram of soil or green tissue, or for each 0.1 gram of dry tissue. Put 100 cc. of distilled water into 200 cc. cylinders (250 cc. Erlenmeyer flasks may be used) and connect the lower ends of the condensing tubes of a distillation battery to glass tubes leading to the bottoms of the cylinders. The Kjeldahl trap arrangement described in the previous paper may be used to assure complete absorption of nitric acid, but since the acid is readily caught in water the traps may be unnecessary. Connect the upper ends of the condensing tubes directly to the Kjeldahl flasks by means of bent glass tubing and rubber stoppers. Set the burners in a pipe that can be readily rotated in order to withdraw all flames instantly. Make the distillations under a hood, since chlorine gas is set free.

When all is ready for immediate connection, wash down the sides of each flask with 25 cc. of 50 per cent by volume sulfuric acid, gently rotating in order to mix without isolating particles of sample on the sides of the flask. Place the flasks on the heating shelf and light all burners with the pipe turned so that the flames do not heat the flasks. Regulate the flames to full height so that the center cones of unburned gases will hit the bottoms of the flasks, while the outer, hot parts will surround the sides, thus preventing the bottoms of the flasks containing the sample and chlorate from heating up more rapidly than the upper parts. (This prevents the accumulation of chlorine peroxide by decomposing it in the hot air above the acid as rapidly as it is formed. All the flames should be of about the same intensity and height.) Now turn the flames on the flasks without connection with the condensers. Heat until violent reaction begins. Withdraw the flames intermittently as long as the action is excessive, but continue the heating as rapidly as possible without too much violence. In this way only traces of chlorine peroxide will form at any one time and there is no danger of an explosion. If the heating is not carried out properly and green fumes collect, take special precaution since chlorine peroxide explodes at about 100°C. (With the Kjeldahl flasks open, however, the explosion is not serious.)

As soon as green fumes cease, withdraw the flames and rapidly connect the flasks to the condensers. Continue the heating carefully until violent reaction is over and bubbling in the receiving flasks ceases. Then distil rapidly until all water is over and the sulfuric acid is clear and begins to reflux on the necks and sides of the flasks. The

* The investigation reported in this paper was made by E. M. Emmert in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.

¹ *This Journal*, 12, 240 (1929).

recurrence of bubbling indicates that the sulfuric acid is beginning to distil. Disconnect the flasks before lowering or withdrawing the flames.

Wash out the condensers into the receivers and boil the distillates several minutes. If cylinders are used, the distillates should be transferred to 250 cc. Erlenmeyer flasks. Make the hot solution up to 150 cc. To an aliquot (usually 50 cc.) containing at least 0.25 mg. of nitrogen, which is the amount giving a good colorimetric reading, add 0.05–0.2 gram of fine solid silver sulfate (depending on the size of the aliquot) while the solution is hot and shake occasionally for 5 minutes. Add 0.5–1.0 gram of calcium hydroxide and shake intermittently for a few minutes. Filter, pouring back until a clear filtrate is secured. The solution should now be practically free of chlorides.

Determine nitrate nitrogen in the solution by the phenoldisulfonic acid method, using sodium hydroxide as the base and adding hydrated lime to the yellow nitrate solution before filtering to read in the colorimeter.

Incomplete Distillation of Ammonia in the Analysis of Ammonium Sulfate¹.

In the direct distillation of ammonia, in the analysis of ammonium sulfate, where the volume in the Kjeldahl flask is approximately 400 cc.

Results of cooperative work on ammonium sulfate.

| TYPE OF BULB | TOTAL VOLUME IN RECEIVER AT END OF DISTILLATION | NITROGEN | AMMONIA | ANALYST |
|--------------|---|-----------------|-----------------|---------|
| | <i>cc.</i> | <i>per cent</i> | <i>per cent</i> | |
| Davisson | 200 | 21.08 | 25.63 | Ellis |
| " | 200 | 20.79 | 25.27 | " |
| " | 200 | 20.70 | 25.17 | " |
| " | 200 | 21.09 | 25.64 | " |
| " | 350 | 21.08 | 25.62 | " |
| " | 350 | 21.17 | 25.74 | " |
| Clark | 200 | 20.82 | 25.30 | " |
| " | 200 | 21.01 | 25.54 | " |
| Davisson | 355 | 21.12 | 25.67 | " |
| " | 360 | 21.19 | 25.76 | " |
| Clark | 280 | 21.20 | 25.78 | " |
| " | 280 | 21.01 | 25.54 | " |
| Davisson | 265 | 21.05 | 25.59 | " |
| " | 370 | 21.12 | 25.67 | " |
| Clark | 390 | 21.12 | 25.67 | " |
| " | 390 | 21.23 | 25.81 | " |
| Davisson | 200 | 21.03 | 25.56 | Clark |
| " | 200 | 21.06 | 25.59 | " |
| " | 350 | 21.06 | 25.59 | " |
| " | 350 | 21.08 | 25.62 | " |
| Clark | 200 | 21.03 | 25.56 | " |
| " | 200 | 21.00 | 25.52 | " |
| " | 350 | 21.08 | 25.62 | " |
| " | 350 | 21.08 | 25.62 | " |

¹ By A. W. Clark, Agricultural Experiment Station, Geneva, N. Y., and H. M. Ellis, Consolidated Gas Co., New York.

to begin with, it was noted that there is incomplete removal of ammonia when the distillate amounts to only 200 cc.

The table shows the variations obtained, as a result of which the following recommendations are offered:

- (1) That a 500 cc. receiving flask be used and a volume of 350 cc. be distilled over.
- (2) That a procedure for the determination of nitrogen in ammonium sulfate be included in the official methods of the association.

BOOK REVIEWS.

Green Manuring. By ADRIAN J. PIETERS, Agronomist in Charge of Clover Investigations, Bureau of Plant Industry, United States Department of Agriculture. 356 pp., 80 figs., 6 x 9 in. John Wiley & Sons, Inc., New York, 1927. Price, \$4.50.

Dr. Pieters has prepared a very comprehensive book on the subject of green manuring, which is valuable for students and scientific investigators, as well as workers in practical agriculture. In the fourteen chapters of the book almost every phase of the green manuring problem is discussed, and the outstanding literature on the subject is reviewed.

Following the introduction, a chapter is given to the history of green manuring, which originated with an ancient practice of the Chinese. In Chapters III, IV and V are discussed the amounts of organic matter in soils, its source and decomposition, and a brief survey is given of the nitrogen problem and nitrogen fixation, all preliminary to the special consideration of green manure crops, their composition and use in Chapters VI, VII and VIII.

Green manuring as a practical farm operation is considered in Chapter IX, and many helpful facts, such as rate of seeding, inoculation, cultivating and fertilizing, are given. A chapter on yields of various crops after green manuring follows.

Possibly the two most valuable chapters, XI and XII, discuss crops used for green manuring and green manuring in the United States. Much valuable information is given in regard to green manures suitable for a large number of crops in various sections of the United States and under varying farm conditions.

A discussion of green manuring in foreign countries is given in Chapter XIII, and the book is concluded with a chapter on economics of green manuring.

The brief summary of each subject given at the conclusion of the chapters is helpful to hurried readers. There are 352 citations to literature, a complete subject index and 80 illustrations.—J. J. SKINNER.

Colloid Chemistry. By JEROME ALEXANDER. 3rd ed. Industrial Chemical Monographs. 254 pages. D. Van Nostrand Co., Inc., New York, 1929. Price, \$3.00.

With the great increase of interest in colloid chemistry a third edition of this book has been prepared and published as one of a series of "Industrial Chemical Monographs". The author has devoted approximately 75 pages to the principles of colloid chemistry in which are included chapters entitled: material units and the forces dominating them; classification of colloids; consequences of subdivision; the ultra-microscope and the general properties of colloids. His explanations of the important principles are very clear for the small amount of space allotted them and in many cases are impressed quite forcibly by homely everyday similes.

From the theoretical section it is only a step into the practical applications of colloid chemistry. The author has delved down into his vast fund of knowledge and shows us the ubiquity of colloid phenomena. He gives us a quick glimpse into more than forty fields in which the principles of colloid chemistry are applied, including such diversified groups as agriculture, photography, chemical analysis, biology and medicine.

An appendix to this edition gives a number of elementary experimental suggestions, all of which can be carried out with readily obtainable apparatus and materials.

The author has condensed into a very limited space the most important principles of colloid chemistry and cited the application of these principles in many industries. From necessity he merely touches the surface of his subject, but for those who wish to go deeper there are numerous references throughout the text, and at the end is found

a bibliography of the important books on colloids. Finally, there is found a glossary which will be of aid to the novice in colloid chemistry, who will also find the subject matter in this book clearly and interestingly presented.—J. R. ADAMS.

Allen's Commercial Organic Analysis, 5th ed., Rewritten and Revised. The organic chemicals and products employed in the arts, manufactures, commerce, medicine science, etc. It treats upon the preparations, modes of analysis, proximate analytical examination; methods for detection and estimation of impurities, adulterations, products of decomposition, etc. In 10 volumes. Vol. 7, *The Vegetable Alkaloids*. Introduction by T. A. Henry; general section on alkaloids by T. M. Sharpe; aconite alkaloids by Francis H. Carr; berberine and its associates by E. Horton; caffeine, tea and coffee by J. J. Fox and P. J. Sageman; cinchona alkaloids by Oliver Chick; cocaine by Samuel P. Sadtler, revised by Norman Evers; cocoa and chocolate by R. Whympster; nicotine and tobacco by R. W. Tonkin; opium alkaloids by Frank O. Taylor; strychnos alkaloids by C. Ainsworth Mitchell; the tropine alkaloids by Francis H. Carr. P. Blakiston's Son & Co., Philadelphia, 1929. Price, \$7.50.

Probably every worker in agricultural chemistry has had occasion to consult some of the volumes of Allen's Commercial Organic Analysis. The introduction to the first edition of "Allen" was written in 1879. In the half century that has since elapsed, organic analysis has made such vast strides that, from a beginning of two volumes with a total of 921 pages, the work has passed through five editions and has already reached seven volumes in this edition with a total of 5210 pages and with 3 more volumes to follow.

Although there are numerous works which deal with toxicology and medical jurisprudence, and which describe the reactions of the alkaloids along with other topics, probably the most important treatises dealing with alkaloids only are those by Pictet¹, Guareschi², Bruhl³, Winterstein and Trier⁴, Wolfenstein⁵, Bauer⁶, Von Korczynski⁷, Henry⁸, and Allen. Although each of these works devotes a part of its attention to the constitution of alkaloids and a part to the physical properties, solubilities and common characteristics upon which the structure of analytical separations is built, the first five are chiefly concerned with the structure of the molecule; consequently the information conveyed by them is not indispensable to the practical analyst. Henry devotes perhaps half of his space to structural studies and the balance to characteristic reactions useful to analysts. The text of Von Korczynski is too brief to be of much value to the practical analyst. This leaves Bauer as almost the only competitor with Allen in the field of books on alkaloids designed preëminently for the use of analysts. For the general use of drug analysts, law enforcement officials and toxicologists, Allen is probably the most useful of all the treatises named.

The botanical source of each alkaloid, its physical and chemical properties, methods for its detection and determination, its pharmacological action and therapeutic uses are discussed in greater or less detail, most attention being given to those properties which are likely to be of the greatest use to analysts. It is evident that the information has been collected with a particular view to its analytical applications. References to original sources of information and to the more highly specialized works are given throughout. The intimate relationships between structure and pharmacological action, a subject of constantly increasing importance, is touched upon but briefly. The value

¹ La Constitution Chimique des Alcaloïdes Végétaux, ed. 2 (1897).

² Einführung in das Studium der Alkaloide, (Translation from the Italian into German by Kunz-Krause) 1896.

³ Die Pflanzen-Alkaloide (1900).

⁴ Die Alkaloide (1910).

⁵ Die Pflanzen Alkaloide (1922).

⁶ Analytische Chemie der Alkaloide (1921).

⁷ Die Methoden der exakten, quantitativen Bestimmung der Alkaloide (1913).

⁸ The Plant Alkaloids, 1924.

of potentiometric methods in titrations is, perhaps, not emphasized as much as the subject merits. In a work of such broad scope some errors are unavoidable. The most serious noted occurs in the discussion on ipecac, in which one of the methods quoted is stated to be that of the German Pharmacopeia of 1926, whereas the method given is taken from the fifth edition of that work. The process for ipecac in the sixth edition is not at all like that in the fifth. This edition of Allen is a worthy addition to the earlier volumes of the series, and it should be in all laboratories where drugs are assayed.—
L. E. WARREN.

NEW BOOKS.

Bread Making. Its Principles and Practice. By E. B. BENNION. 251 pages. Oxford University Press, Amen House, London, E. C. 4, England, 1929. The authors state in the preface, "In presenting this book to the bakery trade an attempt has been made to add a volume to trade literature which will in some measure help to fill the gap which up to now has existed between the small works dealing with general principles and the advanced treatises on the subject of breadmaking and cereal chemistry". Hence such highly technical subjects as hydrogen-ion concentration and colloidal chemistry are treated briefly. Only 10 pages of the appendix are devoted to methods of testing flours, yeast and malt.

Municipal and Rural Sanitation. By VICTOR M. EHLERS and ERNEST W. STEEL. 448 pages. McGraw-Hill Book Co., Inc., New York, N. Y. Price, \$4.00. A useful book to those who are called upon to deal with problems requiring knowledge of sanitation as well as to those working in this field as experts.

Condensed Milk. By ATSUSHI MIYAWAKI. John Wiley and Sons, Inc., New York, N. Y., 1928. Price, \$4.50. The five chapters of this book deal with: (I) The Condensed Milk Industry, (II) Milk Supply, (III) Technology, (IV) Unsweetened Condensed Milk, and (V) Powdered Milk. The author's work in this field of science has not been limited to condensed milk since he has done considerable work in several sections thereof. The book has the advantage of a critical examination by Professor Erf.

The Effects of Moisture on Chemical and Physical Changes. By J. W. SMITH. 44 illus. 8vo. 15 s. net. Longmans, Green and Co., New York, N. Y. As Dr. Donnan says in his preface, "There is perhaps no more interesting and at the same time more puzzling group of phenomena than the chemical and physical effects produced by minute traces of water". The data collected have been grouped under the general headings: Gaseous Reactions, Solid-Gas Reactions, Reactions in Non-aqueous Solvents and The Decomposition of Solids, which comprise approximately half of the book. Theories and other special aspects are fully treated in the remaining half.

ANNOUNCEMENT.

RESEARCH ON IODINE.

Mellon Institute of Industrial Research, Pittsburgh, Pa., has had in operation since January 1, 1928, a Multiple Industrial Fellowship for the investigation of the properties and uses of iodine. This Fellowship, sustained by the Iodine Educational Bureau, 64 Water Street, New York, N. Y., is headed by Dr. George M. Karns, who was formerly a member of the chemical faculty at the University of Illinois. All experimental findings of the Fellowship will be made available to the public.

Recently, through an additional appropriation from the Fellowship donor, Mellon Institute has acted for the Iodine Fellowship in arranging for the study of certain iodine problems in other institutions having special facilities for such types of work. On September 26, 1929, a research grant was made to the Pennsylvania State College, State College, Pa., for a comprehensive investigation, under the direction of Professor E. B. Forbes of the Institute of Animal Nutrition, on the nutritional functions and value of iodine in the feeding of live stock. Although much work has been done on the rôle of iodine in metabolism, especially with reference to the thyroid, little is known in regard to the specific dietetic aspects of this element, particularly in the lower animals. The work that is being carried on by Dr. Forbes and his associates will include studies on cattle, sheep, and swine. The findings of this research also will be reported in the literature, in accordance with the Iodine Educational Bureau's policy of disseminating to every one interested the results of all investigations made under its aegis. Dr. Karns and his co-workers on the Iodine Fellowship at Mellon Institute are cooperating closely with Dr. Forbes and his staff, chiefly by preparing standardized feeds.

Another phase of the iodine research program includes a scholarship founded on October 7, 1929, at the Philadelphia College of Pharmacy and Science by a research grant from the Institute. This scholarship, the work of which is being supervised by Professor Charles H. LaWall, has for its aim a broad investigation of vehicles and solvents for iodine, especially for external use in medicine. Mellon Institute is giving consideration also to the founding of a research scholarship in a medical school for the purpose of aiding in the solution of incompletely answered questions regarding the utility of iodine in internal medicine.



JULIUS BUELL WEEMS, 1865-1930

JULIUS BUEL WEEMS

Dr. Julius B. Weems, chief chemist of the Agricultural Department of the State of Virginia, died suddenly Saturday morning, January 25th, at his home in Ashland, Virginia.

He was in his usual good health and at his office the day previous to his death, and it was a great shock to his many friends and associates in the department, upon arriving for work, to learn that their friend and counselor had passed away.

Dr. Weems was born in Baltimore, Maryland, August 27th, 1865, the son of Edwin Dawson and Rosette Norman Weems. His early education was secured in the public schools of Maryland, and he graduated in 1888 from Maryland Agricultural College with the degree of B. S. In 1888-89 he was instructor in chemistry and mathematics at his alma mater, going from there to Johns Hopkins University, where until 1891 he was a graduate student in chemistry, biology and mineralogy. During the next year he was a chemist at the phosphate mines in Florida. He then attended Clark University at Worcester, Massachusetts, where he was a fellow in chemistry for two years, receiving his Ph. D. in chemistry and pedagogy in 1894.

For the succeeding nine years Dr. Weems was chemist to the Iowa Geological Survey and Professor of Agricultural Chemistry and chemist of the Experiment Station at Iowa State College, Ames, Iowa. Leaving Iowa in 1904, he spent eleven years in investigating farm life and farm problems on a plantation of 2,500 acres. In 1915, he was appointed chief chemist of the Department of Agriculture of Virginia, which position he held until the time of his death. During that time the activities of the department increased about five-fold.

The reputation Dr. Weems attained as a consulting and analytical chemist was much enhanced by his contributions to scientific journals in the form of articles on chemical and agricultural problems, of which he was the author of fifty or more.

He was a regular attendant at the meetings of the Association of Official Agricultural Chemists, having been present at practically all the meetings for the past fifteen years, and under his direction the laboratories of the Division of Chemistry co-operated with the various referees on fertilizers in all collaborative work.

Dr. Weems was a member of the Society of American Bacteriologists, the American Chemical Society, the Association of Official Agricultural Chemists and a fellow of the American Association for the Advancement of Science. Among his personal friends and associates he numbered the Hon. James Wilson, Ex-secretary of Agriculture, Ex-president G. Stanley Hall, of Clark University, and Dr. Ira Remsen, for many years Professor of Chemistry at Johns Hopkins University.

In Worcester, Massachusetts, on June 26th, 1895, he was married to Lila Chapman Fletcher, who survives him with their four children: Rachel Fletcher, now resident physician at Harrisonburg State Teachers College; Carolyn Virginia, who teaches at Lynchburg College; George Macduff, a practising lawyer in Richmond, and Julius Buel, Jr., who is attending high school in Ashland.

Owing to his innate modesty and retiring nature, few were aware of the many accomplishments of Dr. Weems and of the contribution he made to science. Everyone who came in contact with him, however, learned to admire him for his many sterling qualities and to love him for his big-heartedness and genial disposition. He was never too busy to listen to the woes of others and to give advice to those seeking it. The world indeed is a better place for his having lived in it, and his associates, particularly those in the Division of Chemistry, will miss him and mourn his passing for many a day.

W. CATESBY JONES.

EDITORIAL.

DETERMINATION OF BORON IN SOILS.

Recent developments in Western States, particularly California, where minute quantities of boron have wrought serious injury to citrus fruits, have called for a rapid method capable of determining with precision parts per million of this element. Since the association has studied methods for the determination of boron in so many products it is felt that the pressing need of present research problems in connection with soils makes it desirable to add our appeal to that of the committee's which appears as follows in the report of Subcommittee A on Recommendations of Referees for 1929¹: "The Committee repeats the recommendations made last year² relative to the determination of boron and fluorine in soils". Although irrigation water and plant materials are also involved, we wish to direct attention to the determination of the former only in soils.

While this association has accepted official methods³ for foods, waters and fertilizers, no results have been reported as to the applicability of these methods to conditions presented by soils. Hillebrand published the methods for boron which he considered best adapted to rock analysis and made the following statement⁴ with reference to Chapin's⁵ method: "Possibly his procedure may yet prove applicable to rocks as well as to boron minerals". In their most recent work⁶, however, Hillebrand and Lundell have made no changes in this method.

Since Rosenblatt and Gooch⁷ proposed the separation of boron from associated elements by vaporizing the compound of methyl borate from a mixture containing it, modifications have dealt largely with changes in the technic of conducting this separation⁸. The work of Camus and Krumboltz is a distinct departure in procedure, as the former uses acetone to extract boric acid and the latter uses steam for the distillation.

Citations have only been made of a few of the earlier methods used for determining the compound once it is separated from interfering elements, for since Klein⁹ pointed out that a boric acid solution becomes more acidic upon the addition of polyatomic alcohols and sugars there has been an infinite amount of research conducted upon this point. A different procedure was proposed by Bertrand and Agulhon¹⁰, who used curcuma paper and spectroscopic examination of the color imparted to burning hydrogen; by Laval¹¹, who used a paper saturated with a benzine extract of tumeric root; and by others.

We believe the quantitative determination of very small quantities of this element is best accomplished by the spectrophotometric method proposed by Holmes¹², but owing to the expensive apparatus required it would not be of general use. However, by taking a large sample of soil the usual volumetric method should prove accurate enough for practical purposes.

In attempting this determination in soils, it would seem that for the present research would necessarily deal with the separation of boron from the soil, since Funk and

¹ *This Journal*, 13, 57 (1930).

² *Ibid.*, 12, 67 (1929).

³ *Methods of Analysis*, A. O. A. C.

⁴ U. S. Geol. Survey Bull. 700, 235.

⁵ *J. Am. Chem. Soc.*, 30, 1691 (1908).

⁶ *Applied Inorganic Analysis*, 1929, 610.

⁷ Rosenblatt, *Z. anal. Chim.*, 26, 21 (1887); Gooch, *Proc. Am. Acad. Arts Sci.* (1886), p. 167.

⁸ Mandelbaum, *Z. anorg. Chem.*, 52, 364 (1909); Bertrand and Agulhon, *Bull. Soc. Chim.*, 7, 125 (1910); Mannich and Preiss, *Chem. Ztg.*, 32, 314 (1908); Camus, *Anales soc. quim. Argentina*, 2, 123 (1914); Jay and Dupasquier, *Compt. rend.*, 121, 260 (1895); Krumboltz, to be published in *This Journal*.

⁹ *Bull. soc. chim.*, 29, 195 (1878); *Amer. J. Sci.*, 7, 147 (1899); *J. Soc. Chem. Ind.*, 12, 195 (1878).

¹⁰ *Bull. Soc. Chim.*, 7, 90 (1899).

¹¹ *Chem. Ztg.*, 32, 616 (1908).

¹² *This Journal*, 10, 522 (1927).

Winter¹ have shown that in the presence of aluminum, chromium and iron some boric acid remains behind after treatment with methyl alcohol and sulfuric acid, hence this would render a direct determination by distillation, as in the Gooch method, defective. They find that the complete separation of boric acid can be effected if iron and aluminum are precipitated as phosphates from a slightly acid solution or if chromium is hydrolyzed by suitable means to the hydroxide. Since the latter seldom is found in appreciable quantities in soils, the two former elements offer the main obstacle to the determination of boron.

¹ *Z. anorg. Chem.*, **142**, 257 (1925).

FIRST DAY.

MONDAY—MORNING SESSION.

REPORT ON WATERS, BRINE AND SALT.

By C. H. BADGER (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

For some time the following quantitative method has been used in various laboratories of the Food, Drug and Insecticide Administration for the determination of boric acid in water.

BORIC ACID IN WATER.

REAGENTS.

- (a) *Sodium carbonate crystals.*
- (b) *Strong hydrochloric acid.*
- (c) *Tumeric paper.*—Cut in strips 10 inches long and $\frac{1}{2}$ inch wide.
- (d) *Standard boric acid solution.*—Dissolve 1.542 grams of sodium tetraborate (borax) ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) in 1 liter of water. Dilute 100 cc. to 1 liter with water. 1 cc. of the dilute solution = 0.1 mg. of boric acid (H_3BO_3).

DETERMINATION.

Make the sample slightly alkaline with sodium carbonate, if necessary, and adjust to 50 cc. volume in a porcelain evaporating dish either by evaporation or the addition of water. Prepare standards containing, respectively, 0.01, 0.10, 0.50 and 1.0 mg. of boric acid in similar evaporating dishes. Acidify the sample and standards with hydrochloric acid and add 5 cc. to each in excess. Fasten strips of tumeric paper to a rack so that the lower ends dip exactly $\frac{1}{2}$ inch into the solutions. Let stand for 4 hours at room temperature in a place protected from drafts. Compare the characteristic red color developed on the strips of tumeric paper by the sample and standards. Make closer estimations by repeating the determination with a range of standards more nearly equal to the content of boric acid. (It is difficult to compare less than 0.01 mg. or more than 5.0 mg. of boric acid by this method.)

COMMENTS.

While the method described is much more simple in manipulation than the Gooch method referred to on page 104 of *Methods of Analysis*, A. O. A. C., 1925, practice is needed in interpreting the results. However, owing to its simplicity, it is believed that this method or methods similar to it should be studied.

It is recommended¹ that the referee study the method described in this report or similar methods for the quantitative determination of boric acid in waters.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 56 (1930).

No report on tanning materials and leathers was given by the referee.

REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. GRAHAM (Insecticide Control, Food, Drug and Insecticide Administration, Washington, D. C.), Referee.

During 1929 the Referee on Insecticides and Fungicides devoted his attention to methods for the determination of mercury in organic mercurial seed disinfectants.

Three of the methods investigated gave promising results.

The details of the methods are as follows:

I.—Volatilization Method¹.

REAGENTS.

- (a) *Anhydrous sodium carbonate.*
- (b) *Powdered barium carbonate.*
- (c) *Alcohol, 95 per cent.*

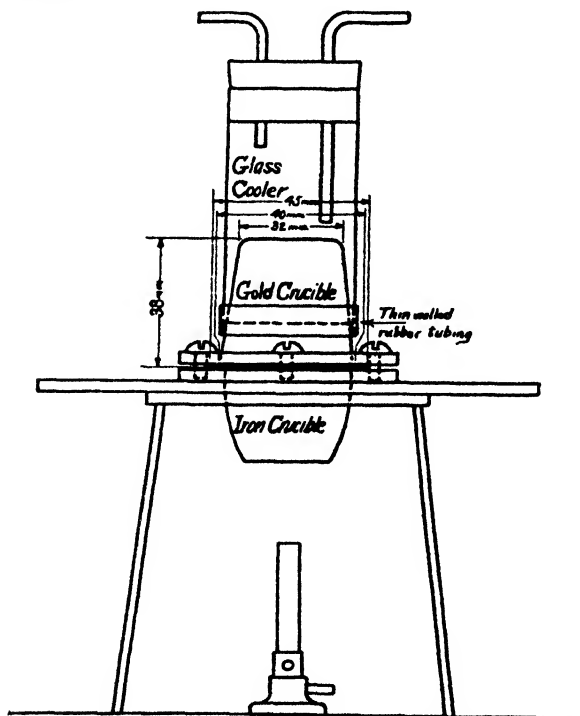


FIG. 1.—APPARATUS TO BE USED IN VOLATILIZATION METHOD.

¹ F. C. Whitmore. Organic Compounds of Mercury, The Chemical Catalog Co., pp. 365-67.

APPARATUS.

The apparatus used (Fig. 1) consists of 2 flanged crucibles that can be clamped mouth to mouth by means of 2 rings and holding screws. The lower crucible is made of iron and sets in an asbestos board with a hole just large enough to receive it; the upper one is of gold, and the opening is slightly larger than that of the other so that there will be no tendency for the mercury to lodge in the joint between the two flanges. The gold crucible is fitted with a cooling device by which water may be slowly circulated through a large tube attached to it by Gooch tubing.

DETERMINATION.

Weigh 1 gram of the sample into the iron crucible and mix it intimately with 5 grams of anhydrous sodium carbonate. Cover the mixture with a thin layer of sodium carbonate and then with 10 grams of finely powdered barium carbonate. Put the weighed gold crucible in place, clamp the two together, set the iron crucible in place in the asbestos board, start the cooling water and gently heat the iron crucible. Do not run the water too fast because the mercury amalgamates best with the gold crucible if the temperature is allowed to rise to about 50°C. Heat below red heat for 30 minutes, cool, remove the gold crucible, wash it with 95 per cent alcohol, dry with the heat of the hand and then place in a calcium chloride desiccator until it attains constant weight. Calculate the increase in weight of the gold crucible as percentage of metallic mercury in the sample. If the product contains more than 12 per cent mercury, less than 1 gram should be used, since 0.12 gram of mercury is about all that the gold crucible can safely retain. Remove the mercury from the gold crucible preparatory to another experiment, by a short ignition at a dull red heat, under a hood having a good draught. The crucible will melt in the full heat of a Bunsen burner.

II.—Precipitation Method.

REAGENTS.

(a) *Hydrogen peroxide*.—A 30 per cent solution, commonly designated as "perhydrol" or "superoxol".

(b) *Potassium permanganate solution*.—An approximately 0.1 N solution.

APPARATUS.

Digestion flask.—A 200 cc. Erlenmeyer flask, fitted with an air condenser by means of a ground-glass joint.

DETERMINATION.

Place 0.5–2.0 grams of the sample, depending on the quantity of mercury present, in the digestion flask. Add 10 cc. of concentrated sulfuric acid, connect the flask to the condenser, and rotate in order to bring all the sample into contact with the acid. Then add 3–5 cc. of the 30 per cent hydrogen peroxide solution, dropwise through the condenser tube, and mix by rotation of the flask. After the active reaction has subsided, heat on a steam bath for 15–20 minutes, add 5 cc. more of the hydrogen peroxide and continue the heating until all organic matter is destroyed (indicated by a clear solution), adding more hydrogen peroxide if necessary. Remove the flask from the bath; wash down the condenser; and transfer the contents to a beaker, filtering if necessary. Dilute to about 200 cc. and destroy the excess of hydrogen peroxide by titration with potassium permanganate. Precipitate the mercury with hydrogen sulfide, filter through a weighed Gooch crucible, and dry the precipitate in the oven at 110°C. Extract the dried precipitate with carbon disulfide to remove any precipitated sulfur, again dry and weigh. From the weight of mercury sulfide calculate the percentage of metallic mercury, using the factor 0.86219.

III.—Titration Method¹.

REAGENTS.

(a) *Standard potassium cyanide solution.*—Dissolve 7 grams of potassium cyanide in water and dilute to 1 liter. Standardize against the 0.05 *N* silver nitrate solution at the time the determination is made, as later described.

(b) *0.05 N silver nitrate solution.*—Adjust to exact 0.05 *N* strength by standardizing against a 0.05 *N* sodium chloride solution containing 2.926 grams of pure sodium chloride per liter.

The other reagents are described under Method II.

APPARATUS.

The apparatus used is the same as that described under Method II.

DETERMINATION.

Place a quantity of the sample containing from 0.2–0.3 gram of mercury in the 200 cc. Erlenmeyer flask fitted with a ground-in condenser. Add 10 cc. of concentrated sulfuric acid and mix by rotating. Close the flask with the condenser and introduce 3–5 cc. of the 30 per cent hydrogen peroxide solution dropwise through the condenser tube, and mix by rotation of the flask. After the active reaction has subsided, heat on a steam bath for 15–20 minutes, add 5 cc. more of the hydrogen peroxide, and continue the heating until all organic matter is destroyed (indicated by a clear solution), adding more hydrogen peroxide if necessary. Remove the flask from the bath, wash down the condenser, rinse the contents into a beaker, and add about 25 cc. of strong ammonium hydroxide or enough to make the solution distinctly ammoniacal. Boil for 3 minutes to destroy Caro's acid, cool, and filter. Then add an excess of the standard potassium cyanide and a few crystals of potassium iodide, and titrate with the 0.05 *N* silver nitrate to a faint turbidity.

Titrate another quantity of potassium cyanide equal to that added to the mercury solution, with the 0.05 *N* silver nitrate. From this titration subtract the number of cubic centimeters of silver nitrate used in the back titration, and the difference will be the number of cubic centimeters of silver nitrate equivalent to the mercury in the sample. From this difference calculate the percentage of mercury on the basis that 1 cc. of 0.05 *N* silver nitrate is equivalent to 0.01 gram of mercury.

The collaborative results obtained on three samples of organic mercurial seed disinfectants are given in Table 1.

Method I appears to be very desirable. It is rapid and easy to carry out, and the results obtained are excellent. On Sample I, the only sample analyzed by more than one analyst, the maximum variation among the results obtained by the three analysts was only 0.1 per cent. The cost of the apparatus is not excessive, although it will usually be necessary to have certain parts of it made to order.

Methods II and III also give good results and should receive further study in view of the fact that all laboratories will not have the apparatus for Method I.

¹ Bauer, *Ber.* 54, Pt. 2, 2079 (1921).

TABLE 1.

*Collaborative results—mercury in organic mercurial seed disinfectants.
(Expressed as percentages of metallic mercury.)*

| ANALYST | SAMPLE | METHOD I | METHOD II | METHOD III |
|---|---------|-------------|--------------|---------------|
| R. Edge Food, Drug and Insecticide Adm. Washington, D. C. | 1 | 5.99 | 5.83 | |
| | | | 5.67 | |
| | Average | | 5.75 | |
| | | | | |
| C. M. Smith Bureau of Chemistry and Soils Washington, D. C. | 1 | 5.98 | | |
| | | 5.98 | | |
| | | 5.94 | | |
| | Average | 5.97 | | |
| J. J. T. Graham | 1 | 6.04 | 5.64 | 6.20 |
| | | 6.00 | 5.66 | 6.18 |
| | Average | 6.02 | 5.65 | 6.19 |
| | 2 | 2.42 | 2.69 | 2.84 |
| | | 2.52 | 2.62 | 2.54 |
| | | . . . | 2.48 | |
| | Average | 2.47 | 2.60 | 2.69 |
| | 3 | 17.76 | 17.74 | 17.68 |
| | | 17.52 | 17.67 | 17.93 |
| | | 17.48 | 17.69 | . . . |
| | Average | 17.59 | 17.70 | 17.81 |

RECOMMENDATIONS¹.

It is recommended—

(1) That the methods for the determination of mercury in organic mercurial seed disinfectants receive further collaborative study.

(2) That the tentative method for copper in Bordeaux-Paris green and Bordeaux-calcium arsenate² be adopted as official (final action). (This method was adopted as tentative at the 1923 meeting³, and was slightly changed and adopted as official, first action, at the 1928 meeting.)

REPORT ON FLUORINE COMPOUNDS.

By G. A. SHUEY (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*.

During the current year work was continued by the associate referee in an attempt to perfect the volatilization method for the determination of fluorine. Considerable time was consumed in conducting preliminary trials for the purpose of arriving at a size and shape of flask that would improve results. These trials resulted in the adoption of a smaller and modified design of the flask used last year.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 56 (1930).

² *This Journal*, 12, 139 (1929).

³ *Ibid.*, 7, 319 (1924).

Many determinations have been conducted under varying conditions, and data have been accumulated. The results, although encouraging, are not consistently accurate, and much remains to be accomplished. Invaluable suggestions offered by C. M. Smith of the U. S. Food, Drug and Insecticide Administration and Howard Allen, Chief Chemist of the Victor Chemical Company of Chicago, are being studied.

It is recommended that study on the volatilization method for the determination of fluorine be continued¹.

REPORT ON CAUSTIC POISONS.

By C. M. SMITH (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

Owing to the fact that during the summer the referee severed his connection with the Insecticide Laboratory of the Food, Drug and Insecticide Administration, in which the analytical work relating to caustic poisons is conducted, no samples of caustic poisons were submitted to collaborators outside that laboratory.

The analytical work necessary for the enforcement of the Federal Caustic Poison Act during the past year has, like that of the preceding year, been practically limited to the estimation of phenol in saponified cresol solutions, coal tar dips, and disinfectants. The method submitted last year continued to give satisfactory results, but it has not been tested further against solutions of known composition. However, the modification of this method, which was developed to take care of interfering methyl salicylate present as a perfume in certain preparations and to which brief reference was made last year, was tested.

The original Chapin method of estimating phenol² is based upon the color reaction between phenol and Millon's reagent, and the modification consists of the previous extraction of the methyl salicylate by means of kerosene. It was found that phenol distributes between water and kerosene in the ratio of 4 to 1 in favor of the former solvent, and this, together with the fact that methyl salicylate is much more soluble in kerosene than in water, explains the efficacy of the modification presented, a detailed description of which follows.

PHENOL.

Modified Chapin Method.—Tentative.

REAGENTS.

(a) *Dilute nitric acid*.—Blow air through strong nitric acid until it is colorless, then dilute 1 volume of this acid with 4 volumes of water.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 56 (1930).

² U. S. Dept. Agr. Bull. 1308 (1924).

(b) *Millon's reagent*.—Treat 2 cc. of mercury in a 200 cc. Erlenmeyer flask with 20 cc. of strong nitric acid. Place the flask under a hood, and after the first violent reaction is over shake vigorously to effect subdivision of the mercury and maintain action. After approximately 10 minutes, when the action has practically ceased even in the presence of undissolved mercury, add 35 cc. of water. If basic salt separates, add sufficient dilute nitric acid to dissolve it. Next add a 10 per cent solution of sodium hydroxide dropwise with thorough mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity. Then add 5 cc. of dilute nitric acid and mix well. Since the solution deteriorates do not use it later than the day following the day of preparation.

(c) *Standard phenol*.—Prepare a stock solution by dissolving a weighed quantity of the pure substance (congealing point not lower than 40°C.) in a sufficient quantity of water to make not less than a 1 per cent solution. From this stock solution make a 0.025 per cent solution in additional distilled water. (This second solution constitutes the final standard and it should be prepared on the day of use.)

(d) *Dilute formaldehyde solution*.—Dilute 2 cc. of commercial 37 per cent formalin solution to 100 cc. with distilled water.

APPARATUS.

(1) *Nessler cylinders*.—50 cc. tall form, matched.

(2) *Test tubes*.—About 180 mm. x 20 mm., provided with rubber stoppers and marked at 25 cc.

(3) *Water bath for heating the test tubes*.—A beaker containing a disk of wire gauze raised slightly from the bottom may be used.

DETERMINATION.

Weigh by difference approximately 10 grams of sample into a separatory funnel (or use 10 cc. and calculate the weight from the density of the sample), add 50 cc. of kerosene, and extract three times with 100 cc. portions of water. Filter the aqueous extracts through a wet filter into a 500 cc. volumetric flask, and make to volume with distilled water.

Transfer a 5 cc. aliquot of this solution to a 200 cc. volumetric flask shortly before the determination is to be carried out, dilute to about 50 cc., add one drop of methyl orange indicator solution and then dilute nitric acid until the solution is practically neutral, make to volume, and shake well.

Place 5 cc. of the diluted solution in each of two of the marked test tubes, and in each of two additional test tubes place 5 cc. of the standard phenol solution (c). Next flow 5 cc. of Millon's reagent (b) down the side of each tube, mix, and place the tubes in a bath of boiling water. Continue the boiling for exactly 30 minutes, cool immediately and thoroughly by immersion in a bath of cold water for at least 10 minutes, and then add 5 cc. of dilute nitric acid (a) to each tube. Mix well, add 3 cc. of dilute formaldehyde solution (d) to one of each pair of tubes, make all the tubes to the 25 cc. mark with water, stopper, shake well, and put aside to stand overnight. The next day the contents of the tubes to which formaldehyde was added will have faded to a yellow, while the others will possess orange or red tints.

Pipet 20 cc. from each of the two phenol tubes and transfer to 100 cc. volumetric flasks, treat each with 5 cc. of the dilute nitric acid, make to the mark, and mix. (The red flask contains the "phenol standard", and the yellow flask the "phenol blank".) Transfer these solutions to burets. Pipet 10 cc. of each sample solution into Nessler tubes. The orange or red one constitutes the "unknown" and the yellow one the "sample blank", and each Nessler tube must be distinctly marked to avoid confusion. Next add to the sample blank tube a measured quantity of phenol standard from its

buret and add the same volume of phenol blank to the unknown, thoroughly agitate, and compare the colors. When the tubes have been brought to a match, each cc. of the phenol standard employed is equivalent to 1 per cent phenol if a portion of sample weighing exactly 10 grams was used.

In using this method the following precautions should be borne in mind. A pair of phenol tubes affords sufficient final solutions for assaying several unknowns, but all the latter must have accompanied the phenol solutions throughout the entire process with identical reagents and treatment. If the end point has been inadvertently overrun, it is possible to work back to it; but, since mistakes are easy to make in this procedure, it is better to repeat the comparison on fresh portions from the original tubes. Too much delay in matching the tubes must be avoided once the titration has been started, otherwise the excess of formaldehyde left in the blanks may have time after mixture to affect the intensity of the red color.

Millon's reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used.

The method just described was tested¹ against five solutions containing known percentages of phenol, *p*-cresol, kerosene, and birch oil (methyl salicylate). Chemically pure phenol and a commercial grade of birch oil were used. The *p*-cresol, of which a technical grade boiling at 202°C. and containing only 0.2 per cent of phenol was used, was added to increase the solubility of the phenol. The results obtained are shown in the following table.

| SOLUTION NO. | COMPOSITION | | | | PHENOL FOUND |
|-----------------|-----------------|-----------------|------------------|-----------------|-----------------|
| | Phenol | Oil of Birch | <i>p</i> -Cresol | Kerosene | |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1 | 5 | 10 | 5 | 80 | 5.2, 5.1 |
| 2 | 10 | 10 | 10 | 70 | 10.0, 10.1 |
| 3 | 15 | 10 | 15 | 60 | 14.8, 14.8 |
| 4 | 10 | 5 | 10 | 75 | 10.2, 10.2 |
| 5 | 10 | 20 | 10 | 60 | 10.2, 10.2 |

DISCUSSION.

These results are seen to be very favorable, in view of the fact that an accuracy of 1 part in 10 is all that is to be expected from a colorimetric procedure such as this. The quantities of methyl salicylate used were

¹ This work is described more fully by E. H. Hamilton and C. M. Smith in *Ind. Eng. Chem., Anal. Ed.*, 1, 232 (1929).

much in excess of any which are likely to be encountered in commercial samples.

Since there appears to be no reason why this method should not also be applicable to preparations containing no methyl salicylate, no need for separate procedures is apparent in the two cases, and the method here described can be used on all types of samples with the assurance that if present methyl salicylate will cause no trouble.

It is suggested that future work be directed along the lines outlined last year—the development of methods for the estimation of “free or chemically unneutralized” acids and especially for “free and chemically uncombined” ammonia, since they comprise 7 of the 12 substances classified as poisons by the Caustic Poison Act.

RECOMMENDATIONS¹.

It is recommended that the modified Chapin method described in this report be adopted for the estimation of phenol (carbolic acid) in such products as cresol, saponified cresol solutions, coal tar dips, disinfectants, fly sprays, and other similar preparations suspected of coming within the provisions of the Caustic Poison Act.

REPORT ON SOILS AND LIMING MATERIALS.

By W. H. MACINTIRE (Agricultural Experiment Station, Knoxville, Tenn.), *Referee*.

Other than participation with the Associate Referee for Liming Materials in studies that have been offered for publication, the work of the general referee has been in an advisory capacity. The former Associate Referee on Reaction Value of Alkaline Soils, P. S. Burgess, was unable to continue his work. No one could be secured to take his place.

The referee concurs in the recommendations of the Associate Referee for Liming Materials and in those of the Associate Referee on Copper, Manganese and Zinc in Soils.

It is recommended² that a study be made to determine the neutralizing possibilities of the calcium-silica combinations that occur in materials that are offered for sale as “soil amendments”.

No report on the reaction value of alkaline soils was given. No associate referee was appointed.

No report on the reaction value of acid soils was given by the associate referee.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 56 (1930).

² For report of Subcommittee A and action of the association, see *This Journal*, 13, 57 (1930).

REPORT ON LIMING MATERIALS.

By W. M. SHAW (Agricultural Experiment Station, Knoxville, Tenn.),
Associate Referee.

During the past year the Associate Referee for Liming Materials has made a further study of the lime-silica reactions. The Associate Referee and the General Referee have offered for publication a paper¹ entitled "The Nature of Calcium Hydroxide Absorption by Hydrated Silica", from which the following summary is taken.

1. The system $\text{CaO-SiO}_2\text{-H}_2\text{O}$ was investigated as a part of a fundamental and major study of the soil's components that react with aqueous $\text{Ca(OH)}_2\text{-CaSO}_4$ solutions.

2. The literature discloses few systematic investigations as to the nature of the reactions between Ca(OH)_2 and hydrated silica.

3. Preliminary work on the speed of Ca(OH)_2 absorption by silica of different origin and degree of hydration with agitation and attrition demonstrated that:

a. The degree of hydration of an amorphous silica has little effect on the speed of the CaO-SiO_2 reaction.

b. The speed of the reaction (and attainment of equilibrium) decreases with increase in the size of the particles of silica gel.

c. Continuous agitation with $\frac{1}{8}$ -inch steel balls was found to be the most expeditious procedure to induce maximum absorption with particles of 35- to 200-mesh fineness.

d. The abrasive action of the attrition agent on the glass containers and the enhanced CaO-SiO_2 reactions thus induced were studied.

4. The absorption of Ca(OH)_2 by hydrated silica was first investigated by the use of dialyzed silica hydrosol and two kinds of silica gels, which led to the development of the formula $x = 2.34C^{.1818}$, in which x represents the molar ratio CaO/SiO_2 in the absorption product and C the final Ca(OH)_2 concentration. This formula is limited in its application to equilibrium concentrations above 0.005 N .

5. The experiments on the reaction between Ca(OH)_2 and silica disclosed a series of hydrated calcium silicates of varying degrees of solubility and varying CaO/SiO_2 ratios, whose existence is governed by the total available $\text{Ca(OH)}_2\text{/SiO}_2$ ratio and the resulting OH-ion concentration.

6. The solubility of their respective silica contents in dilute hydrochloric acid may be used to differentiate between the "absorption-product" and the "solid-phase reaction-product" that result from $\text{Ca(OH)}_2\text{-SiO}_2$ reactions.

It is again recommended that in the next edition of *Methods of Analysis* provision be made to insure against the error introduced by hydrogen sulfide in the determination of carbonate carbon dioxide.

REPORT ON LESS COMMON METALS IN SOILS.

By J. S. MCHARGUE (Department of Chemistry, Agricultural Experiment Station, Lexington, Ky.), Associate Referee.

A method for the determination of manganese in soils was adopted last year. During this year further experiments were made to ascertain whether the bisulfate fusion method could be modified to include deter-

¹ Complete paper to appear in *Soil Science*.

minations of copper and zinc in addition to manganese. As a preliminary test the following experiments were made:

Three 5-gram portions of finely ground soil were weighed separately and transferred into three 50 cc. platinum crucibles, and 12.5 grams of finely powdered potassium bisulfate was added and thoroughly mixed with each of the portions of the soil. The crucibles, with their lids, were placed over a Bunsen burner and heated gently at first and the heat was gradually increased until the crucibles and lids were red hot and the contents had ceased to froth. The time required for a single fusion was about 25 minutes. After fusion was completed the molten contents were spread over the inner walls of the crucibles by rotating them in a horizontal position. When partially cooled the crucibles and lids were carefully placed in separate beakers containing about 50 cc. of dilute hydrochloric acid, digested over holes on a hot water bath, and stirred occasionally with a glass rod. The contents of the crucibles soon disintegrated and dissolved, after which the crucibles and their lids were removed and rinsed into the respective beakers. The contents of the three beakers were rinsed into a porcelain dish and evaporated to dryness. The dry residue was moistened with 15 cc. of hydrochloric acid (1 + 1), and enough hot distilled water was added to bring the soluble salts into solution with stirring. The insoluble residue of sand and silica was filtered on a small size Büchner funnel and thoroughly washed with hot water, suction being used. The filtrate was transferred to a 250 cc. Erlenmeyer flask, diluted to a volume of about 200 cc. and heated to near the boiling point; a slow stream of hydrogen sulfide gas was passed through the solution for about 15 minutes while it was rotated, after which the flask was stoppered tightly and set aside in a warm place overnight. The dark brown colored precipitate of copper sulfide was filtered on a pad of paper pulp and washed with a dilute (2.5 per cent) solution of hydrochloric acid saturated with hydrogen sulfide gas. The pad of paper pulp containing the copper sulfide was transferred to a porcelain crucible and ignited until all the carbon was consumed and the copper sulfide was burned to copper oxide. The copper oxide was digested with a few drops of hydrochloric acid on the hot water bath, and any insoluble residue was filtered off and washed. The filtrate was made alkaline with a few drops of ammonia, and if a precipitate formed it was filtered out and washed. The filtrate was transferred to a small porcelain dish and made acid with nitric acid and evaporated to dryness, and the residue was heated on a sand bath to decompose ammonium nitrate. The residue of copper oxide was digested in a few drops of nitric and hydrochloric acid, and the excess of hydrochloric acid was expelled by evaporation two or more times with a drop or two of strong nitric acid. The copper nitrate was slightly acidified with a very small drop of dilute nitric acid, transferred to a 50 cc. volu-

metric flask and made to the mark. Copper was determined in an aliquot as described in the method for the determination of this element in plants¹.

MANGANESE.

Five cubic centimeters of strong nitric acid was added to the filtrate from the copper sulfide and boiled until ferrous iron was oxidized to the ferric condition as shown by the solution changing to a brownish-yellow color. The solution was cooled and made to a volume of 200 cc., an aliquot was taken and evaporated with sulfuric acid until chlorides were expelled and manganese was determined by the colorimetric periodate method described for manganese in plants.²

Results obtained with the method, expressed in percentage.

| | | COPPER | MANGANESE | ZINC |
|---------------------|-------|--------|-----------|--------|
| Soil No. 1 | | | | |
| a..... | | 0 0021 | 0.0130 | 0.0075 |
| b..... | | 0 0019 | 0 0131 | 0.0109 |
| c..... | | 0 0018 | 0.0130 | 0.0130 |
| Average..... | | 0.0019 | 0.0130 | 0.0105 |
| Cu, Mn and Zn added | | | | |
| d..... | | 0 0072 | 0 0375 | 0.0210 |
| e..... | | 0 0072 | 0.0375 | 0.0230 |
| f..... | | 0.0076 | 0.0375 | 0.0230 |
| Average..... | | 0.0073 | 0.0375 | 0.0223 |
| Soil No. 2 | | | | |
| | grams | | | |
| a..... | 5 | 0.0020 | 0.2700 | 0.0104 |
| b..... | 5 | 0.0020 | 0.2700 | 0.0094 |
| c..... | 10 | 0.0023 | 0.2700 | 0 0113 |
| d..... | 15 | 0.0028 | 0.3000 | 0.0115 |

ZINC.

An aliquot representing 10 grams of soil was transferred to a beaker, diluted to 250 cc., heated to near the boiling point, and iron and alumina were precipitated by adding a slight excess of ammonia. A spoonful or two (depending on the bulk of the precipitate) of paper pulp was transferred into the beaker containing the precipitate and stirred until the paper pulp was uniformly distributed with the precipitate, after which the mixture was filtered on a small Büchner funnel and washed thoroughly with hot water, suction being used. The filtrate was concentrated to about 50 cc., transferred to an Erlenmeyer flask, and acidified with acetic acid; and the solution was saturated with hydrogen sulfide and set aside overnight. The zinc sulfide was filtered on a pad of paper pulp, washed with a dilute solution of ammonium acetate saturated with hydrogen sulfide. The pad of paper pulp containing the zinc sulfide

¹ *This Journal*, 12, 35 (1929).

² *Ibid.*, 36.

precipitate was burned in a porcelain crucible to zinc oxide and cooled, and the zinc oxide was dissolved in a few drops of dilute hydrochloric acid, filtered into a 50 cc. volumetric flask, diluted to the mark, and mixed thoroughly. Zinc was determined in an aliquot as described in the method for the determination of zinc in plants¹.

It is recommended² that further collaborative work be done on the proposed methods for the determination of copper, manganese and zinc in soils and that consideration be given to the possibility of determining arsenic, iron, titanium, nickel and cobalt by these methods.

No report on feeding stuffs was given by the referee.

REPORT ON STOCK FEED ADULTERATION.

By H. E. GENSLER (Department of Agriculture, Harrisburg, Pa.),
Associate Referee.

During the year the associate referee submitted samples to a number of collaborators, requesting them to make determinations for the amount of hoof according to the method devised by W. F. Sterling³. The response was poor, and results came in so late that it was decided that the study of this method should be continued.

The associate referee examined specimens of hoof meal, "raw" and treated, with the Hanovia Ultraviolet Quartz Lamp, and found that the fluorescence given off by this material was so definite and characteristic that when it was mixed with meat scrap it was quite possible to detect the presence of the meat. Various quantities of hoof were added to the meat scrap and detected, even though 1 per cent only was present. He also made a similar study of dried buttermilk and found that this substance was readily detected, even when only traces were present.

The results of this study are regarded as quite significant and important, since they show the possibilities of a new form of examination for the identification of ingredients in feeding stuffs.

It is recommended² that the study of the Sterling method for the determination of hoof be continued.

¹ *This Journal*, 12, 36 (1929).

² For report of Subcommittee A and action of the association, see *This Journal*, 13, 57 (1930).

³ *This Journal*, 12, 129 (1929).

REPORT ON MINERAL MIXED FEEDS.

By H. A. HALVORSON (State Dairy and Food Department, St. Paul, Minn.), *Associate Referee*.

At the meeting of this association last year, the Associate Referee on Mineral Mixed Feeds was instructed to continue the study of methods for the determination of lime (CaO) and iodine.

Owing to the amount of work involved, it was not possible to carry out all the recommendations approved. However, considerable progress was made in the study of Olson's modification for the determination of lime, and a great deal of work was done by the collaborators with a method for the determination of iodine proposed by Knapheide and Lamb¹.

Two samples of known composition, resembling commercial mineral feeds, were sent to twenty-three collaborators who had expressed a willingness to assist in the work. Of this number, nineteen reported results of the determination of lime in both samples, and eleven collaborators submitted results of determinations of iodine.

Sample No. 1 consisted of the following ingredients and proportions: Tankage 10 per cent, spent bone black 30 per cent, ground limestone 40 per cent, salt (sodium chloride C. P.) 19.9 per cent, potassium iodide 0.1 per cent. Determinations of calcium oxide in each ingredient by the method under consideration showed that the tankage furnished to the mixture 1.16 per cent, the spent bone black 14.47 per cent, and the limestone 22.32 per cent, making a total of 37.95 per cent. If it is assumed that the method used was satisfactory and the individual determinations were correct, and also that there was no calcium oxide in the salt and potassium iodide, the actual amount of calcium oxide in sample No. 1 is 37.95 per cent. From the percentage of potassium iodide used in the mixture, it will be seen from calculation that the sample contained 0.0764 per cent of iodine.

Sample No. 2 was made up of the following ingredients: Tri-basic calcium phosphate (pure precipitated) 40 per cent, calcium carbonate (precipitated) 40 per cent, salt (sodium chloride C. P.) 19.96 per cent, potassium iodide 0.04 per cent. Determinations of calcium oxide in the same manner as described in the preparation of sample No. 1 showed that the calcium phosphate furnished to this mixture 19.37 per cent and the calcium carbonate 22.37 per cent, making a total of 41.74 per cent calcium oxide or lime in sample No. 2. Based on the percentage of potassium iodide used, sample No. 2 contained 0.0306 per cent iodine. The instructions sent to all the collaborators contained the details for determining calcium oxide in mineral feeds published in the report of

¹ *J. Am. Chem. Soc.*, 50, 2121 (1928).

TABLE 1.

Calcium oxide in A. O. A. C. mineral feed samples.

| COLLABORATORS | SAMPLE NO. 1 37.95% CALCULATED FROM ANALYSIS OF INGREDIENTS | | | | SAMPLE NO. 2 41.74% CALCULATED FROM ANALYSIS OF INGREDIENTS | | | |
|--|---|-----------------|---|-----------------|---|-----------------|--|-----------------|
| | Using HCl | | Using Acetic Acid | | Using HCl | | Using Acetic Acid | |
| | Indi- vidual | Aver- age | Indi- vidual | Aver- age | Indi- vidual | Aver- age | Indi- vidual | Aver- age |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| W. P. Elmslie and M. D. Knapheide Moorman Mfg. Co. Quincy, Ill. | 37.23 37.44 37.50 37.50 | 37.42 | 37.30 37.30 37.43 37.36 | 37.35 | 41.76 41.82 41.33 41.22 | 41.53 | 41.66 41.62 41.26 41.30 | 41.46 |
| W. D. Richardson Swift & Co., Chicago Analyst "A" | 37.25 37.18 | 37.21 | 37.25 37.25 | 37.25 | 41.62 41.62 | 41.62 | 42.04 41.90 | 41.97 |
| Analyst "B" | 37.33 36.82 | 37.07 | 36.78 37.21 | 36.99 | 41.82 41.74 | 41.78 | 41.89 41.96 | 41.92 |
| Analyst "C" | 36.72 36.87 | 36.79 | 37.01 37.14 | 37.07 | 42.18 42.18 | 42.18 | | |
| W. L. Latshaw and E. J. Coulson Manhattan, Kan. | 38.22 38.09 | 38.15 | | | 41.70 42.00 | 41.85 | | |
| J. W. Bowen Purina Mills, St. Louis | 37.38 37.38 37.37 37.46 37.53 | 37.42 | 37.21 37.24 37.23 37.42 37.39 | 37.30 | 41.92 41.96 41.94 41.64 41.71 | 41.83 | 41.87 41.91 41.88 41.78 41.66 | 41.82 |
| R. O. Baird and C. S. Ladd Bismarck, N. D. | 37.45 37.40 37.45 37.40 | 37.43 | 37.40 37.45 37.45 37.45 | 37.44 | 40.59 40.50 40.72 40.80 | 40.65 | 40.50 40.59 40.80 40.88 | 40.69 |
| E. L. Redfern Des Moines, Ia. | 35.91 35.91 | 35.91 | 35.14 34.89 | 35.01 | 44.04 43.73 | 43.88 | 44.04 44.14 | 44.09 |
| L. E. Bopst College Park, Md. | 39.90 39.76 | 39.83 | 39.90 40.04 | 39.97 | 44.10 44.36 | 44.23 | 44.38 44.24 | 44.31 |
| H. D. Spears and W. G. Terrell Lexington, Ky. | 37.81 37.60 37.70 37.74 | 37.71 | 37.60 37.41 37.74 37.74 | 37.62 | 40.36 40.75 41.15 40.96 41.15 41.11 | 40.91 | 41.09 41.90 41.03 40.80 41.28 41.04 | 41.19 |
| J. W. Kellogg Harriaburg, Pa. | 37.45 37.58 37.37 37.45 | 37.46 | | | 42.08 41.80 41.80 41.70 | 41.84 | | |

TABLE 1.—Continued.
Calcium oxide in A. O. A. C. mineral feed samples.

| COLLABORATORS | SAMPLE NO. 1 37.95% CALCULATED FROM ANALYSIS OF INGREDIENTS | | | | SAMPLE NO. 2 41.74% CALCULATED FROM ANALYSIS OF INGREDIENTS | | | |
|--|---|--------------------------|----------------------------------|--------------------------|---|--------------------------|----------------------------------|--------------------------|
| | Using HCl | | Using Acetic Acid | | Using HCl | | Using Acetic Acid | |
| | Indi- vidual | Aver- age | Indi- vidual | Aver- age | Indi- vidual | Aver- age | Indi- vidual | Aver- age |
| W. B. Griem and LaVerne Clifcorn Madison, Wis. | <i>per cent</i> 36.56 | <i>per cent</i> 36.56 | <i>per cent</i> 36.86 | <i>per cent</i> 36.86 | <i>per cent</i> 40.20 | <i>per cent</i> 40.20 | <i>per cent</i> 40.21 | <i>per cent</i> 40.21 |
| Mayne R. Coe Washington, D. C. | 37.81 37.38 37.81 | 37.67 | | | 41.63 41.63 41.35 | 41.54 | | |
| H. H. Hanson and R. Earle Dicky Dover, Del. | 39.12 38.83 38.97 | 38.97 | 38.97 39.27 38.97 38.83 | 39.01 | 41.76 42.49 42.49 42.64 | 42.35 | 41.91 42.49 42.20 42.05 | 42.16 |
| G. S. Fraps College Station, Tex. | 37.76 | | | | 40.67 | | | |
| W. F. Walsh Geneva, N. Y. | 37.29 37.57 37.29 | 37.38 | 37.43 37.43 37.36 | 37.41 | 41.36 41.50 41.64 | 41.50 | 41.64 41.64 41.78 | 41.69 |
| A. O. Olson St. Paul, Minn. | 37.65 37.66 | 37.66 | 37.50 37.77 | 37.64 | 42.28 42.14 | 42.21 | 42.14 42.21 | 42.18 |
| G. G. Frary Vermillion, S. D. | 37.50 37.50 37.45 35.70 | 37.54 | 36.90 37.10 | 37.00 | 40.80 41.23 40.65 40.67 | 40.84 | 41.10 41.44 | 41.27 |
| Kraybill and Yund Lafayette, Ind. | 37.90 37.94 | 37.92 | 37.31 38.08 | 37.69 | 42.42 42.28 | 42.35 | 42.56 42.00 | 42.28 |

the associate referee for 1926¹. Each collaborator was requested to determine lime in both samples by the method as printed, and also to determine lime by a slight modification of the method. The modification consisted of making the solution acid with acetic acid, instead of 0.1 *N* hydrochloric acid, and omitting the procedure of bringing the solution back to the neutral point with 0.1 *N* sodium hydroxide. It was thought that time could be saved by the use of this modification.

Collaborators were requested to express their preferences for either one or the other of these procedures. Most of the collaborators concluded that both were satisfactory. A comparison of the results reported by both procedures indicates, in most instances, that there is little to

¹ *This Journal*, 10, 177 (1927).

choose between them except the possibility of saving a little time. In the opinion of the associate referee, it will be advisable to have the method stand as first published and make it optional to use the acetic acid modification when desired.

Table 1 shows results reported by the collaborators, both as to individual determinations and averages. The individual figures are given because they show the possibility of obtaining closely agreeing results. Because of the satisfactory work done this year and on the 1927 samples, it is the opinion of the associate referee that this method for the determination of calcium oxide in mineral feeds should be adopted as tentative.

The accurate determination of iodine in mineral feeds offers many difficult problems. The method published in the report of the associate referee in 1926 was tried out in 1927, and while the results were not accurate, most of the collaborators who reported were able to find at least half of the iodine that had been added to the samples. It was thought that additional work on this method would prove valuable. Since Knapheide and Lamb had been very successful in determining iodine, it was decided to put their method to a thorough trial this year and not to attempt additional work on the method suggested by Griem in 1926.

The instructions on the iodine determination sent to the collaborators were accompanied by a full reprint of the method. In addition, there were sent four paragraphs of precautions and further details, which the associate referee had obtained from the authors. The results of the reporting collaborators are shown in Table 2.

As will be seen from Table 2, eleven collaborators assisted in this part of the work. In some cases the results are very good, in others they are just as disappointing as the results obtained in 1927. Most of the collaborators complained of the tediousness of the method and of the many difficulties encountered. Several thought that the method was unduly long and complicated and that it might be shortened and simplified by further study. It is evident from the results reported that experience either with this method or in work of a similar nature is an important factor in obtaining good results.

In the opinion of the associate referee, work on methods proposed for the determination of iodine in mineral feeds should be continued. Since the subject is one to which analysts are giving much attention, it might be well for the present not to restrict the work to any specific method, but to allow the associate referee to choose such methods for further collaborative work as might be called to his attention. Manufacturers of products in which iodine is used are especially interested in finding an accurate, suitable and quick method for the determination of this ingredient. With this interest on the part of state analysts and with the cooperation of manufacturers, a satisfactory method is certain to be devised.

TABLE 2.

Iodine in A. O. A. C. mineral feed samples.

| COLLABORATORS | SAMPLE NO. 1—0.0764 % ADDED | | SAMPLE NO. 2—0.0306 % ADDED | |
|--|--|-----------------|--|-----------------|
| | Individual | Average | Individual | Average |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| W. P. Elmslie and M. D. Knapheide | 0.0785 0.0797 0.0750 0.0772 0.0730 0.0717 | 0.0758 | 0.0311 0.0284 0.0290 0.0314 0.0324 | 0.0305 |
| W. D. Richardson Analyst "A" | 0.066 0.072 | 0.0690 | 0.026 0.027 | 0.0265 |
| Analyst "B" | 0.055 0.056 0.067 | 0.0593 | 0.021 0.021 | 0.0210 |
| W. L. Latshaw and E. J. Coulson | 0.359 0.289 | 0.3240 | 0.110 0.089 | 0.0995 |
| H. D. Spears and W. G. Terrell Modification of 1927 Method | 0.0654 0.0680 | 0.0667 | 0.0275 0.0279 | 0.0277 |
| J. W. Bowen | 0.103 0.107 0.106 0.112 | 0.1070 | 0.036 0.035 0.036 0.036 0.026 0.027 | 0.0327 |
| R. O. Baird and C. S. Ladd | 0.0753 0.0737 0.0707 0.0750 0.0740 0.0738 0.0766 0.0751 | 0.0743 | 0.0121 0.0122 0.0111 0.0126 0.0102 0.0107 0.0077 0.0081 | 0.0105 |
| J. W. Kellogg | 0.0643 | 0.0643 | 0.0146 | 0.0146 |
| W. B. Griem and F. L. Gunderson | 0.054 | 0.0540 | 0.034 | 0.0340 |
| Mayne R. Coe | 0.40 0.40 0.35 0.38 | 0.3825 | 0.18 0.16 0.12 0.15 | 0.1525 |
| G. G. Frary | 0.045 0.066 0.060 | 0.0570 | 0.016 0.018 0.019 | 0.0177 |

RECOMMENDATIONS¹.

It is recommended—

(1) That the proposed method for the determination of lime in mineral feeds be adopted as tentative and that the acetic acid modification of this method be optional, also that further work be done on this method.

(2) That further work on the determination of iodine in mineral feeds be carried on by methods proposed for consideration.

(3) That methods for the determination of iodine in organic minerals be studied.

REPORT ON MOISTURE DETERMINATION IN FEEDING STUFFS.

By G. E. GRATTAN (Department of Agriculture, Ottawa, Canada),
Associate Referee.

Work on the comparison of the official vacuum oven method with the 135°C. air oven method was continued. This method is as follows:

Regulate an electric air oven to 135°C., $\pm 2^\circ$. Using low, covered, aluminum dishes, weigh approximately 2 grams of the sample into each dish and shake until the contents are evenly distributed. With the covers removed, place the dishes and covers in the oven as quickly as possible and dry the samples for 2 hours. After placing the covers on the dishes, transfer them to a desiccator to cool, and weigh. Calculate the loss in weight as moisture.

TABLE 1.
Collaborative results.

| OFFICIAL VACUUM METHOD. | | | |
|--|---------------------------|-----------------------|--------------------|
| LABORATORY | OIL CAKE MEAL per cent | MEAT MEAL per cent | SHORTS per cent |
| 1 | 8.50 | 5.70 | 11.90 |
| 2 | 9.04 | 6.23 | 12.78 |
| 3 | 8.83 | 5.81 | 12.17 |
| 4 | 8.97 | 5.94 | 12.38 |
| 5 | 8.93 | 5.98 | 12.40 |
| Average | 8.81 | 5.89 | 12.25 |
| Mean difference | 0.54 | 0.53 | 0.88 |
| ELECTRIC AIR OVEN, 2 HOURS AT 135°C. | | | |
| 1 | 9.19 | 6.17 | 12.68 |
| 2 | 9.17 | 6.41 | 14.21* |
| 3 | 8.95 | 6.06 | 12.82 |
| 4 | 9.02 | 6.43 | 13.53 |
| 5 | 9.44 | 6.52 | 13.65 |
| Average | 9.15 | 6.31 | 13.17 |
| ELECTRIC OVEN IN REFEREE'S LABORATORY. | | | |
| | 8.69 | 5.54 | 12.07 |
| | 8.56 | 5.83 | 12.03 |
| | 8.67 | 5.59 | 12.04 |

* Omitted from average.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 57 (1930).

Samples of linseed oil cake, meat meal and shorts were sent to several laboratories. Reports were received from five of them. They are shown in Table 1.

The difference between the official vacuum oven method average and the proposed method as reported by the collaborators is 0.3, 0.5, and 0.9 per cent, respectively, which would appear to be within experimental error.

It is recommended¹ that the electric air oven method be adopted tentatively as an alternative method to be used when a vacuum oven is not available.

REPORT ON SUGARS AND SUGAR PRODUCTS.

By R. T. BALCH (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

Active work during the year was conducted by the Associate Referees on Honey, Maple Products, Polariscopic Methods, and Chemical Methods for Reducing Sugars. The Associate Referee on Drying, Densimetric and Refractometric Methods was unable to make the investigations outlined and approved at the last session and hence will present no report. The association was again unable to find anyone willing to undertake work on starch conversion products.

The Associate Referee on Honey has been critically investigating methods for the selective determination of levulose in the presence of dextrose for the purpose of establishing the levulose-dextrose ratio for authentic honeys. Until a suitable method is found, collaborative work on this subject is being held in abeyance. As being of interest to the association, the referee wishes to report that the Carbohydrate Division of the Bureau of Chemistry and Soils is undertaking a thorough study of the diastase content of honeys to determine genuineness, owing to the importance placed upon this test by European countries that import large quantities from this country. The Fiehe reaction is also being considered, and the results will doubtless be of considerable value to the association, since Nelson's modification of this test is recommended for collaborative study.

Attention was given by the Associate Referee on Maple Products to a collaborative study of the official and modified methods for determining the lead number, with particular reference to maple sirup. His recommendations for further study are concurred in by the referee.

In collaborative work on polariscopic methods, the associate referee has substantiated past investigations, which showed that the invertase method is the only dependable one that can be used for the determina-

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 58 (1930).

tion of sucrose in mixtures of invert sugar, reversion products and amino compounds. The inconsistent results obtained by the collaborators during the past two years with the acid inversion methods on sugar mixtures in the presence of amino compounds suggest the occurrence of some uncontrolled reaction between amino acids and the reducing sugars under the conditions of sucrose inversion. The associate referee has consented to give this subject a critical study before continuing collaborative work with these methods on the analysis of sugar products containing amino compounds. It is planned, however, to conduct collaborative studies on the effect of lead clarification on the various Clerget methods as recommended and approved by the association in 1927.

The Associate Referee on Chemical Methods for Reducing Sugars has been devoting his attention to a critical study of various methods for determining reducing sugars in an effort to improve the accuracy of existing methods as well as to determine the suitability of the more recent methods for official use. He recommends that further study be made before submitting them to collaboration or approval by the association, and the referee concurs.

REPORT ON HONEY.

By H. A. SCHUETTE (Department of Chemistry, University of Wisconsin, Madison, Wis.), *Associate Referee.*

The associate referee has no formal report to offer on collaborative work done on honey during the past year. This is in line with the recommendation made at the last meeting of the association¹ to the effect that no further attempts be made in the establishment of a levulose-dextrose ratio for pure honey until satisfactory methods can be found for the determination of these sugars when both are present in the material under examination.

To that end a critical study was made of Nyns'² suggestion that, under closely guarded experimental conditions, Ost's³ solution will show preference for a ketose sugar in the presence of an aldose. That this is not necessarily the case, as Jackson has pointed out⁴, and that there exists an hitherto unsuspected source of error in the use of cupro-potassium carbonate solutions for the determination of reducing sugars, has been made the subject of a separate communication.

¹ *This Journal*, 12, 158 (1929).

² *Sucr. Belge*, 44, 210 (1924).

³ *Ber.*, 23, 1035, 3003 (1890); 24, 1634 (1891); *Chem.-Ztg.*, 19, 1784, 1829 (1895).

⁴ *This Journal*, 12, 168 (1929).

RECOMMENDATIONS¹.

It is recommended that Nelson's² modification of the Fiehe test³ for the detection of artificial invert sugar in honey be made the subject of collaborative study.

REPORT ON MAPLE PRODUCTS.

By J. F. SNELL (Macdonald College, Province of Quebec, Canada),
Associate Referee.

The collaborators will be designated as follows:

- A—Name withheld, New York City.
- B—Sam Byall, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C., under direction of R. T. Balch.
- E—E. E. Massey, Macdonald College, P. Q.
- G—Name withheld, New York City.
- K—Roland E. Kremers, Postum Company, Inc., Battle Creek, Mich.
- M—F. S. Morison, Cary Maple Sugar Company, St. Johnsbury, Vt.
- O—W. O. Winkler, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C., under direction of J. W. Sale.
- S—Lev Skazin, Macdonald College, P. Q.
- W—J. M. Widmer, Penick and Ford, Ltd., Cedar Rapids, Iowa.
- Z—J. G. Van Zoeren, De Pree Laboratories, Inc., Holland, Mich.

Table 1 gives the description of the sirups.

Ten collaborators reported (1) on the dry matter of sirup as prepared for analysis and on the Canadian lead number (2) by the tentative method and (3) by the Fowler modification⁴, in which the lead precipitate is washed with *cold* water. Nearly all also reported lead numbers by modifications of the Fowler method in which the quantity of basic acetate solution used was reduced from 2.0 cc. to, respectively, (4) 1.5 cc. and (5) 1.0 cc. Ash values and electrical conductivity were reported less completely.

DRY MATTER.

The variability of the dry-matter content of sirups prepared for analysis according to the official method was surprising. As shown in Table 2, the average obtained in the 20 sirups by different analysts (omitting G) varied from 57.4 to 65.3 per cent, and only two obtained results approximating 65, the percentage desired. The average difference between the highest and lowest dry matter percentage found in the sirups prepared by the same analyst was 7.7 per cent, and the minimum (by analyst A) was 5.8. O's minimum (55.2) was 11.5 below his

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 58 (1930).

² *This Journal*, 12, 323 (1929).

³ *Methods of Analysis*, A. O. A. C., 1925, 201; *Z. Nahr. Genussm.*, 15, 492 (1908); 16, 75 (1908).

⁴ Fowler and Snell, *Ind. Eng. Chem., Anal. Ed.*, 1, 8-12 (1929).

TABLE 1.

Description of maple sirups.

(All these sirups were produced in the Province of Quebec.)

| NO. | COUNTY | SOIL | TREES | RUN | FLAVOR | CLARITY | COLOR * |
|-----------|---------------------------|---------------------------|-------------|--------|--------------------------------|-------------------|---------|
| 1 | Compton | Rolling, slaty | Hard | Early | Sweet, sickish, not pleasant | Clear | 6 |
| 2 | Compton | Rolling, slaty | Hard | Middle | Sweet, not pleasant | Clear | 6 |
| 3 | Compton | Rolling, slaty | Hard | Late | Sweet, musty | Sl. cloudy, sed't | 8 |
| 4 | Huntingdon | Very low, loam and gravel | 65% soft | First | Good | Clear | 10 |
| 5 | Huntingdon | Gravel | Hard, young | First | Off-flavor present | Sediment | 7 |
| 6 | Huntingdon | Gravel and loam | A few soft | First | Harsh, slightly moldy | Slightly turbid | 9 |
| 7 | Huntingdon | Gravel | Hard | Middle | Good, mild | Sediment | 8 |
| 8 | Huntingdon | Gravel and loam | A few soft | Middle | Good, slightly flat | Clear | 12 |
| 9 | Huntingdon | Gravel | Hard | Late | Good | Clear | 10 |
| 10 | Huntingdon | Very low, loam and gravel | 65% soft | Late | Slightly sour and musty | Clear | 11 |
| 11 | Huntingdon | Gravel and loam | A few soft | Late | Slightly sour and vinous | Slight sediment | 11 |
| 12 | Beauce | Stony | | Late | Smoky, sour | Opaque | 13 |
| 13 | (St. Philibert) Beauce | Stony | | Middle | | | 8 |
| 14 | (St. Philibert) Beauce | Stony | | First | Rich, slightly stale | Slightly turbid | 7 |
| 15 | (St. Philibert) Beauce | Stony | | First | Sweet, not characteristic | Clear | 9 |
| 16 | (St. Martin) Beauce | Black | | Middle | Sweet, slightly harsh, woody | Opaque | 11 |
| 17 | (St. Martin) Beauce | Black | | Late | Moderate, slightly harsh | Clear | 13 |
| 18 | (St. Martin) Beauce | | | Last | Heavy, slightly sour | Opaque | 13 |
| 19 | Vaudreuil Beauce | Rocky and mountainous | Hard | Early | Sweet, moderate, maple, mellow | Clear | 10 |
| 20 | Beauce | Rocky and mountainous | | Late | Acrid-sour, sorghum-like | Nearly opaque | 14 |
| Observer: | | Collectors | | K | | K | S |

* Bryan Scale.

TABLE 2.
Canadian lead values—average on all sirups by different methods.

| ANALYST | S | O | G | K | Z | B | A | W | M | E | MEAN |
|--|------|------|-------|-------|------|------|------|------|------|--------|------|
| Sirups analyzed | 20 | 20 | 20 | 20-19 | 20 | 20 | 19 | 20 | 20 | 6 | |
| Average % D. M. | 61.8 | 62.5 | 56.1* | 64.5 | 65.3 | 57.4 | 61.4 | 59.0 | 61.0 | 63.7 | |
| Average result by: Tentative method | 3.46 | 3.25 | 3.24 | 3.25 | 3.20 | 3.60 | 3.74 | 3.05 | 3.68 | (3.18) | 3.40 |
| Fowler method 2 cc. | 3.77 | 3.38 | | 3.45 | 3.50 | 3.67 | 3.86 | 3.51 | 3.88 | (3.37) | 3.62 |
| Cold method 1.5 cc. | 3.87 | 3.45 | | | 3.55 | 3.74 | 4.00 | 3.43 | 3.93 | (3.46) | 3.71 |
| Cold method 1.0 cc. | 3.88 | 3.29 | | | 3.40 | 3.55 | 3.81 | 3.15 | 3.81 | (3.53) | 3.56 |

* Not boiled to 104°.

TABLE 3.
Canadian lead values—variation of duplicates in different methods—average of all sirups.

| ANALYST | S | O | G | K | Z | B | A | W | M | E | MEAN |
|--|-------|-------|-------|-------|-------|-------|-------|-------|---------------|-------|-------|
| Sirups analyzed | 20 | 20 | 20 | 19 | 19 | 20 | 8-9 | 10 | | 6 | |
| Average difference between maximum and minimum: | | | | | | | | | No Duplicates | | |
| Tentative method | 0.058 | 0.129 | 0.121 | 0.087 | 0.087 | 0.044 | 0.079 | 0.063 | | 0.147 | 0.094 |
| Fowler 2 cc. | 0.037 | 0.106 | | 0.068 | 0.052 | 0.049 | 0.160 | 0.065 | | 0.065 | 0.074 |
| Cold, 1.5 cc. | 0.034 | 0.086 | | | 0.071 | 0.069 | 0.189 | 0.092 | | 0.075 | 0.068 |
| Cold, 1.0 cc. | 0.027 | 0.091 | | | 0.068 | 0.067 | 0.129 | 0.086 | | 0.068 | 0.077 |

maximum, and his second lowest was 7.7 below the maximum. W's minimum (54.7) was 9.6 below his maximum.

It is evident, then, that the present directions for preparing samples for analysis are inadequate to secure uniformity in total solids content of the sirups. No doubt greater precision could be obtained by boiling to a definite Abbé refractometer reading or to a definite density (to indicate which a suitable glass bulb might be used in the hot liquid).

One of the collaborators, who is of the opinion that the invert sugar content has a material influence on the lead value, suggests that the sirups be prepared to a definite content of total sugars as determined by inverting and making a reducing sugar analysis. He writes: "I have generally found that the total sugars in good maple sugar will run close to 90 per cent, but if you have a poor maple sugar, one which has been burned, the sugar content will drop through caramelization. I have seen the sugar content of badly burned sugars drop down to 75 and 76 per cent". This view is based on 10 years' experience, not on any systematic investigation. It should be checked by such investigation.

The same collaborator reported invert sugar in the 20 sirups, and certainly no correlation appears between the lead numbers and the reducing sugars. Sirup No. 12, which gave the highest lead numbers, was found to contain 5.2 per cent, while No. 20, with the next highest lead numbers, had 13.3 per cent invert sugar. The sirups with lowest lead numbers, viz., Nos. 1, 4, 6 and 15, had, respectively, 2.7, 4.3, 23.5 and 1.4 per cent of invert sugar. (No. 6 was moldy. See Table 1.)

LEAD VALUES.

The values obtained in the four modifications of the Canadian lead method were compared in respect to (1) magnitude, (2) variations between duplicates by the same analyst, (3) variations in the mean results of the different analysts, and (4) range of value among the 20 sirups.

(1) *Magnitude.*

In Table 2 are given the averages of the lead values obtained by each analyst on all the sirups analyzed by him according to each of the four methods. In every case higher values were obtained with the Fowler than with the tentative method, and with one exception higher values were obtained with 1.5 than with 2.0 cc. of reagent.

No correlation appears between the magnitude of the lead values and the total solids content of the prepared sirups.

(2) *Variations between duplicates by the same analyst.*

Table 3 gives the mean differences between the maximum and minimum lead values obtained by each observer by each of the variations of the lead method. In general, closer duplicates were obtained by the

TABLE 4.

Canadian lead values—range among the sirups expressed as percentage of the mean value found by the analyst.

| ANALYST | S | O | G | K | Z | B | A | W | M | E | MEAN |
|-----------------------|------|------|------|------|------|------|------|------|------|------|------|
| Tentative method..... | 97.4 | 84.7 | 69.4 | 86.8 | 95.7 | 87.5 | 92.5 | 91.8 | 96.2 | | 89.1 |
| Fowler 2 cc..... | 93.9 | 85.5 | | 84.1 | 90.0 | 88.8 | 90.1 | 66.1 | 92.3 | | 86.4 |
| Cold, 1.5 cc..... | 82.8 | 72.8 | | | 77.2 | 74.9 | 76.5 | 58.0 | 78.9 | | 74.4 |
| Cold, 1.0 cc..... | 62.6 | 51.4 | | | 61.1 | 60.6 | 57.2 | 42.2 | 57.5 | | 56.1 |

TABLE 5.

Electrical conductivity values.

| | S | M | W | E | ALL* |
|------------------------------|-----|-----|-----|-------|------|
| Sirups analyzed..... | 20 | 20 | 10 | 5 | |
| Maximum found in number..... | 12 | 12 | 18 | (3) | |
| Minimum found in number..... | 1 | 1 | 1 | (2) | |
| Average value..... | 148 | 136 | 161 | (128) | 148* |
| Maximum value..... | 192 | 177 | 198 | (139) | 198 |
| Minimum value..... | 111 | 109 | 125 | 117 | 109 |
| Range..... | 81 | 68 | 73 | 22 | 89 |
| Range (% of average)..... | 55 | 50 | 45 | | 60 |

* Excluding E, whose measurements were all on sirups of low conductivity.

cold-washing methods, though the results of collaborators A and B are exceptional in this respect. Among the three cold-washing methods, four collaborators out of seven obtained closer results with the use of 2.0 than by the use of 1.5 or 1.0 cc. of reagent; two (S and A) had the closest agreement with 1.0 cc., and one (O) with 1.5 cc. Averaging the results of all collaborators on a basis of equality, those obtained by the Fowler method as published show the least variability of duplicates. If only the results of the four collaborators who reported on 19 or 20 sirups by all four methods are included, average variations of 0.080, 0.061, 0.065 and 0.063 are noted by the four respective modifications of the lead method. It would appear that the substitution of a cold-washing method for the present tentative method might result in the attainment of greater precision.

(3) *Variations between the mean results on identical sirups by the different analysts.*

Using the Canadian lead method, the different analysts obtained values on individual sirups differing by from 15.9 (in the case of Sirup No. 12) to 47.7 (No. 2) per cent of the mean of the values found by all. The average difference between the highest and lowest values reported on the individual sirups was 0.94, which is 27.8 per cent of the average lead value of the twenty sirups (3.40).

In the Fowler method, the values obtained on individual sirups varied by 10.9 (No. 16) to 40.4 (No. 2) per cent of the mean for the given sirup. The average difference by this method (0.72) was 20.0 per cent of the average value of the twenty sirups (3.62).

In the cold-washing method in which the quantity of reagent used was reduced to 1.5 cc. the corresponding figures were: 8.0 (No. 8) to 36.7 (No. 2) per cent; average $0.79 = 21.1$ per cent of the mean (3.71). Where the quantity of reagent was lowered to 1.0 cc. the figures were: 13.8 (No. 6) to 39.8 (No. 12) per cent; average $0.86 = 23.7$ per cent of the mean (3.56).

It would, therefore, appear probable that better concordance amongst analysts would be reached if a cold-washing method were employed.

Certain analysts tended to get higher and certain others lower lead values by all the methods. Thus, in all the sirups except one the maximum Canadian lead value was obtained by one or other of the three analysts, A, E and M. By the Fowler method, the maximum fell to one of these same three analysts in 18 of the 20 sirups and by the 1.5 cc. method in 19 out of 20. By the 1.0 cc. method, S got the maximum value in 8 sirups, tying with A in two instances, but in the other 12 sirups the maximum fell to either A, E or M—usually to A. On the other hand, analysts O, W and Z provided 15 of the minimum results by the Canadian

TABLE 6.

Ash weights (percentage on dry matter).

| | TOTAL | | | | | SOLUBLE* | | | | | INSOLUBLE | | | | |
|---------------------------------|-------|-------|------|-------|------|----------|-------|------|-------|------|-----------|------|------|------|------|
| | S | A | M† | W | All | S | A | M† | W | All | S | A | M | W | All |
| Sirups analyzed..... | 13 | 19 | 20 | 9‡ | | 13 | 18§ | 20 | 9‡ | | 13 | 17 | 20 | 10 | |
| Maximum in number..... | 12 | 12 | 12 | 12‡ | | 12&13 | 19 | 12 | 18 | | 9 | 12 | 12 | 12 | |
| Minimum in number..... | 4 | 1 & 2 | 2 | 4 | | 1 | 4 | 2 | 4 | | 15 | 19 | 15 | 15 | |
| Average value..... | 1.00 | 1.00 | 0.95 | 1.05‡ | 1.00 | 0.61 | 0.58§ | 0.60 | 0.69‡ | 0.62 | 0.40 | 0.42 | 0.35 | 0.35 | 0.38 |
| Maximum value..... | 1.35 | 1.46 | 1.32 | 1.54‡ | 1.54 | 0.81 | 0.82 | 0.82 | 0.96 | 0.96 | 0.56 | 0.68 | 0.50 | 0.70 | 0.70 |
| Minimum value..... | 0.79 | 0.81 | 0.67 | 0.72 | 0.67 | 0.44 | 0.35 | 0.34 | 0.43 | 0.34 | 0.26 | 0.20 | 0.26 | 0.21 | 0.20 |
| Range..... | 0.56 | 0.65 | 0.65 | 0.82 | 0.87 | 0.37 | 0.47 | 0.48 | 0.53 | 0.62 | 0.30 | 0.48 | 0.24 | 0.49 | 0.50 |
| Range, per cent of average..... | 56 | 65 | 68 | 78 | 87 | 61 | 81 | 80 | 77 | 100 | 75 | 113 | 69 | 140 | 132 |

* By difference.

† No duplicate determinations.

‡ Results for total ash (1.78) and soluble ash (1.53) on No. 20 omitted because so far from those of other collaborators as to suggest an undetected error.

§ Result on No. 15 (0.22) omitted because comparison with other collaborators suggests that those for soluble and insoluble ash may have been interchanged.

|| Result on No. 15 (0.61) omitted for reason stated in Note †, and that on No. 4 (0.55) as too far out of line with those of other collaborators (0.28, 0.29 and 0.29).

method, 17 by the Fowler method, 18 by the 1.5 cc. and 19 by the 1.0 cc. method.

Besides personal differences, two factors suggest themselves as possibly contributing to the tendency of the analysts to get higher or lower results in this determination. One is the variations in the water content, to which the sirups were prepared. As already stated, there appears to be no definite correlation here. The other is the lead subacetate solution, which may have varied in the different laboratories. A comparison of the solutions would have been of interest in this connection.

(4) *Range among the 20 sirups.*

It will be seen in Table 4 that the cold-washing methods give results exhibiting a narrower range of values in the genuine sirups than the tentative method and that the range becomes narrower as the quantity of basic acetate solution employed decreases.

Sirup No. 12 gave the highest lead value, by all the methods, in the hands of all the analysts except one (W). With 1 cc. of reagent this analyst obtained a higher result (3.79) on No. 3 than on No. 12 (3.59), and his results, in general, were low compared with those of the others. On 9 of the 20 sirups he obtained lower lead values by the tentative method, on 5 by the Fowler method, on 10 by the 1.5 cc. method and on 13 by the 1.0 cc. method than did any of the other collaborators. His result on No. 12 by the 1.0 cc. method was 0.72 lower than the next lowest, while on No. 3 it was only 0.09 lower than the next. In the other methods his work, like that of the other analysts, placed No. 12 highest. As regards the sirups showing the lowest values, results were less concordant. By the tentative method, five analysts found No. 4 the lowest, two No. 6, one No. 1 and one No. 2; by the Fowler method, five No. 4, two No. 6 and one No. 1; by the 1.5 cc. method, four No. 4, one No. 1, one No. 6 and one No. 18; and by the 1.0 cc. method, three No. 4, three No. 18 and one No. 1. Variation in the preparation of the sirups for analysis may affect the results obtained in the lead value determination. The amount of calcium malate removed by filtration may depend not only on the degree to which the sirup is concentrated, but also on the time it is maintained at temperatures near its boiling point. Calcium malate crystallizes slowly even from simple aqueous solution. A more important factor influencing the lead value results is doubtless the basicity of the lead subacetate solution, and in future collaborative work it would be well to have all the solutions used analyzed in the same laboratory.

ELECTRICAL CONDUCTIVITY.

Determinations of conductivity value were made by two of the collaborators (S and M) on all 20 samples, by another (W) on 10 representa-

TABLE 7.
Ash alkalinities (milliliters of 0.1 N per 100 grams dry matter).

| | TOTAL* | | | | | SOLUBLE | | | | | INSOLUBLE | | | | |
|----------------------------------|--------|-------|-------|-------|-------|---------|------|------|------|------|-----------|------|------|------|------|
| | S | A | M† | W | All | S | A | M† | W | All | S | A | M† | W | All |
| Sirups analyzed..... | 13 | 19 | 20 | 10 | | 13 | 19 | 20 | 10 | | 13 | 19 | 20 | 10 | |
| Maximum in number. | 12 | 12&20 | 12 | 12 | | 12 | 17 | 12 | 18 | | 12 | 12 | 12 | 12 | |
| Minimum in number..... | 4 & 15 | 19 | 4 | 4 | | 1 | 9 | 1 | 4 | | 15 | 19 | 15 | 4 | |
| Average value..... | 177.4 | 172.4 | 172.6 | 161.2 | 170.9 | 74.1 | 74.5 | 81.3 | 76.7 | 76.7 | 103.3 | 97.9 | 91.3 | 84.5 | 94.3 |
| Maximum value..... | 252 | 207 | 241 | 242 | 252 | 102 | 106 | 107 | 100 | 107 | 150 | 134 | 134 | 144 | 150 |
| Minimum value..... | 140 | 141 | 137 | 103 | 103 | 58 | 52 | 62 | 52 | 52 | 66 | 56 | 62 | 51 | 51 |
| Range..... | 112 | 66 | 104 | 139 | 149 | 44 | 54 | 45 | 48 | 55 | 84 | 78 | 72 | 93 | 99 |
| Range (per cent of average)..... | 63 | 38 | 60 | 86 | 88 | 59 | 72 | 55 | 63 | 72 | 81 | 79 | 79 | 110 | 105 |

* By addition.
† No duplicate determinations.

tive samples, and by a third (E) on Nos. 2-5. All the results obtained by M were lower and those obtained by W, with one exception, were higher than those obtained by S. The average value for the samples according to M was 136, according to S, 148, and according to W it was 161. (See Table 5.) On the 10 samples analyzed by all three, the average values were: M 140, S 151, and W 161. The difference between M's and W's average for these 10 samples is 13.9 per cent of the mean (151) of the three.

If 1 sample of the 20 is omitted, M's results on individual sirups are from 5 to 13 per cent below those of S, and if 2 out of the 10 are omitted, W's results are from 6 to 13 per cent above those of S. It would appear as if differences in standardization of the cell might be largely responsible for the variations in the results of the different analysts. Even as it is, the average difference between the results of different analysts on individual sirups is less than the corresponding figure for any of the other methods examined in this work.

The range of conductivity value among the 20 sirups is also less than that of either the lead or the ash values.

ASH VALUES.

Table 6 summarizes the quantities and Table 7 the alkalinities of the ash—total, water-soluble and insoluble. There is often a very considerable divergence between the results of different analysts on the same sirup. On this account, as will be seen by reference to the notes on Table 6, a few of the figures reported have been excluded in averaging and in stating the maxima and minima. Nevertheless, such divergences in the directly determined values remain as those between 0.67 and 0.85, 0.93 and 1.14, 0.85 and 0.98 in the percentage of total ash; 0.30 and 0.42, 0.33 and 0.49, 0.31 and 0.44 in the percentage of insoluble ash; 52 and 81, 73 and 107, 82 and 100 in the alkalinity of the soluble ash; and 73 and 123, 52 and 74, 69 and 115 in the alkalinity of the insoluble ash. In the alkalinities differences in the standardization of the solutions used in titration or of the depth of indicator color adopted as the end point may account for some analysts reporting lower results than others on all or nearly all samples. (W's results, for instance, average lower than M's for both alkalinities and are individually lower on almost all samples). The quantities of standard alkali and acid solutions actually used in the titrations are small, and the experimental errors are considerably magnified in calculating from an actual 3.25 grams to a basis of 100 grams of dry matter.

The variations in the ash weights reported by different analysts are ascribable in part to the fact that the total ash is very hygroscopic, and the insoluble ash slightly so. The introduction of special precautions in weighing might minimize errors due to this cause. In weighing the

ash the associate referee uses a sheet of tin plate as a cover to the platinum dish and a similar piece as a counterpoise. Analyst S notes the time required to weigh the first of a pair of duplicates, then puts on the weights for the second one before placing the dish on the balance pan, weighs quickly, allows to stand for the same length of time as was consumed in weighing the first, weighs again, and corrects his first duplicate accordingly.

COMPARISON OF METHODS.

In Table 8 the values obtained by the various modifications of the lead method, the various ash values and the conductivity values are compared with regard to their variability among the 20 sirups and in the hands of the different analysts. Comparisons are made in three ways, which are described in the notes to the table, and the figures obtained for each method in these three ways are averaged for a final comparison. It will be noted that with very few exceptions the methods rank in the same order on bases B and C as on basis D, and that on basis A the chief difference is that the percentage and alkalinity of the total ash rank above the alkalinity of the soluble ash as regards constancy. In a comparison of a large number of published analyses according to basis C, made by the associate referee 10 years ago¹, the most constant values—with the exception of the conductivity, which easily ranks first—were found to be the total ash and the alkalinity of the soluble ash. The alkalinity of the total ash was not considered on that occasion. The results of the present work suggest that it ought to be given consideration in future.

TABLE 8.
General comparison of the analytical values studied.

| VALUE | BASIS OF COMPARISON* | | | |
|---------------------------------------|----------------------|----|-----|----|
| | A | B | C | D |
| Conductivity value | 11 | 53 | 60 | 41 |
| Alkalinity of soluble ash | 19 | 62 | 72 | 51 |
| 1.0 cc. lead value | 24 | 56 | 85 | 55 |
| Alkalinity of total ash | 16 | 62 | 88 | 55 |
| Percentage of total ash | 13 | 67 | 87 | 56 |
| 1.5 cc. lead value | 21 | 74 | 96 | 64 |
| Percentage of soluble ash | 25 | 75 | 100 | 67 |
| 2.0 cc. (Fowler) lead value | 20 | 86 | 110 | 72 |
| Alkalinity of insoluble ash | 23 | 87 | 105 | 72 |
| Canadian lead value | 28 | 89 | 122 | 80 |
| Percentage of insoluble ash | 28 | 99 | 132 | 86 |

* A.—The difference between the highest and the lowest results obtained by different analysts on the same sirup was divided by the mean of the results obtained on that sirup by all analysts and multiplied by 100. The twenty results so obtained were then averaged.

B.—The difference between the highest and the lowest values found in all the sirups by each analyst was divided by the average value obtained in all by the same analyst and multiplied by 100. The results for the various analysts were then averaged.

C.—The difference between the highest and the lowest values found by any analyst in any sirup was divided by the average of the average values found in all the sirups by the different analysts.

D.—The average of A, B, and C.

SUMMARY.

1. The percentage of dry matter in maple sirups prepared for analysis by the official method varies widely even in the hands of the same analyst.

2. All collaborators obtained higher lead numbers by the Fowler modification of the Canadian lead method (cold washing) than by the present tentative method (hot washing).

3. In the cold washing procedure eight collaborators out of nine obtained on the average higher lead values by the use of 1.5 cc. of the prescribed solution of basic lead acetate per 5 grams of dry matter of sirup than by the use of 2.0 cc.

4. Six out of eight collaborators obtained closer agreement of duplicates with the cold-washing than with the hot-washing method.

5. Among the 20 sirups examined the range of variation of the values obtained by the cold-washing methods is in general less than that of the present Canadian lead value, and it becomes less when the quantity of reagent used is decreased (down to 1.0 cc.).

6. The conductivity value is by far the least variable of the values studied.

7. Wide variations exist in the ash results obtained by different analysts on identical sirups.

8. The least variable of the ash results are the alkalinities and percentages of soluble and total ash. Increased precision in weighing and in the standardization of solutions would no doubt further decrease the variations of these values.

9. The lead values obtained by cold-washing methods are less variable than the percentage or the alkalinity of the insoluble ash.

ACKNOWLEDGMENT.

The expense of collecting and distributing the samples was met out of a grant from the National Research Council of Canada for research on maple sugar and sirup.

RECOMMENDATIONS¹.

It is recommended—

(1) That the method of preparation of the sample be studied with a view to attaining closer agreement in results in dry matter content.

(2) That to the official method for the determination of total ash², the following words be added: "taking precautions to guard against, or to correct for³, absorption of moisture during weighing".

(3) That the following paragraph be inserted after 109:

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 58 (1930).

² *Methods of Analysis*, A. O. A. C., 1925, 203.

³ See this report.

"Alkalinity of the Total Ash—Official.

Add the alkalinities of the soluble and insoluble portions **108** and **109**."

(4) That the misprint in **112** (second) "with 50 cc. of water" be corrected to read "with 500 cc. of water".

(5) That further collaborative study of the modified Canadian lead method be made, such study to include a comparison of the lead subacetate solutions used by the collaborators, and also further study of the relative advantages of the use of 1.0, 1.5 and 2.0 cc. of reagent.

(6) That in the directions for conductivity value, the words "and multiply by 10^{-6} " in **114** and "and multiply by 10^6 " in **115** be deleted.

(7) That the tentative method for conductivity value as so amended be adopted as official (first action).

(8) That the advisability of altering the quantity of sirup to be taken for the conductivity value (**115**) from 22 grams to 25 grams, so that the same solution may be used for Canadian lead value and conductivity value be given consideration.

No report was given on starch conversion products. No associate referee on this subject was appointed.

No report on drying, densimetric, and refractometric methods was given by the associate referee.

REPORT ON POLARISCOPIC METHODS.

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*.

The recommendations offered by the associate referee for work to be done in 1928¹ provided that the four inversion methods under investigation be applied to mixtures of pure sugars with pure amids and amino acids. Research along this line was carried out in 1928², but during the course of the analyses it developed that the invert sugar sirup prepared from sucrose by means of invertase was not suitable for the purpose for which it was intended. For this reason results were reported only for sucrose, for a commercial invert sirup, and for a mixture of the two, with or without the further addition of asparagine or aspartic acid. Experiments with invert sugar were postponed until the present year, and it was decided to use a mixture of equal parts of dextrose and

¹ *This Journal*, **11**: 175 (1928).

² *Ibid.*, **12**: 158 (1929).

levulose instead of the invert sirup prepared from sucrose. The dextrose was kindly furnished by R. F. Jackson, of the Bureau of Standards; it was a sample of exose, from the Corn Products Refining Co., and was over 99.9 per cent pure. The levulose was prepared in the Bureau of Chemistry and Soils. The analytical work was done by Sam Byall in the Bureau of Chemistry and Soils, and by C. A. Gamble and J. E. Mull in the New York Sugar Trade Laboratory. The writer gratefully acknowledges the cooperation of these men.

The following working plan was adopted:

The products to be used in this year's investigation are:

A.—Sucrose (Domino tablet sugar). It should give a direct polarization of at least 99.85.

B.—Dextrose, of high purity.

C.—Levulose, of high purity.

D.—A solution containing in 100 cc. total volume 2.6 grams of l-aspartic acid plus the required quantity of sodium hydroxide to bring the pH of the solution to 7.

E.—A solution containing in 100 cc. total volume 2.6 grams of l-asparagine plus the required quantity of sodium hydroxide to bring the pH of the solution to 7.

Use the concentrations given below, for both the direct and invert polarization:

1. Sucrose, 13 grams to 100 cc.
2. Sucrose, 13 grams, plus 10 cc. of solution D, to 100 cc.
3. Sucrose, 13 grams, plus 10 cc. of solution E, to 100 cc.
4. Dextrose, 6.5 grams, plus levulose 6.5 grams, to 100 cc.
5. Dextrose, 6.5 grams, plus levulose 6.5 grams, plus 10 cc. of Solution D, to 100 cc.
6. Dextrose, 6.5 grams, plus levulose 6.5 grams, plus 10 cc. of Solution E, to 100 cc.
7. Sucrose 6.5 grams, plus dextrose 3.25 grams, plus levulose 3.25 grams, to 100 cc.
8. Sucrose 6.5 grams, plus dextrose 3.25 grams, plus levulose 3.25 grams, plus 10 cc. of Solution D, to 100 cc.
9. Sucrose 6.5 grams, plus dextrose 3.25 grams, plus levulose 3.25 grams, plus 10 cc. of Solution E, to 100 cc.

Use the following methods of analysis:

(a) Official invertase method at room temperature, *Methods of Analysis*, A. O. A. C., 1925, p. 185, 22 (a) and (b) with one modification: Use 10 cc. of invertase of $k = 0.1$.

(b) Official acid method at room temperature, *Methods of Analysis*, A. O. A. C., 1925, p. 187, 23 (c).

(c) Jackson and Gillis method No. II (U. S. Bur. Standards Sci. Paper 375, p. 182 (g), and pp. 184-185).

(d) Jackson and Gillis method No. IV (U. S. Bur. Standards Sci. Paper 375, p. 182 (g), and pp. 187-188).

The concentrations given above under 1 to 9 are those to be used for the actual readings in the polariscope. Do not dilute the solutions any further. In other words, the inverting agents and the chemicals used in the Jackson and Gillis methods must be added to the materials named under 1 to 9 in the same 100 cc. flask.

Make the solutions to be used for the direct reading nearly to the mark and allow to stand overnight to complete mutarotation.

Also allow the solutions to be used for the invert reading to stand overnight, at about 26°-27°C.

Make the readings in a water-jacketed tube at 20°C.

Carry out all determinations in duplicate, and if the results do not check within 0.1 degree, run a repeat.

TABLE 1.

Sucrose.

| ANALYST, AND METHOD USED | DIRECT POLARIZATION | INVERT POLARIZATION | PERCENTAGE |
|---|------------------------|------------------------|-------------|
| Invertase method | | | |
| S. Byall | 49.93 | -16.12 | 50.00 |
| C. A. Gamble, J. E. Mull | 49.95 | -16.08 | 49.98 |
| | | | <hr/> 49.99 |
| A. O. A. C. acid method | | | |
| S. Byall | 49.93 | -16.67 | 50.00 |
| C. A. Gamble, J. E. Mull | 49.95 | -16.55 | 49.93 |
| | | | <hr/> 49.97 |
| Jackson and Gillis method No. II | | | |
| S. Byall | 49.78 | -16.92 | 50.03 |
| C. A. Gamble, J. E. Mull | 49.78 | -16.92 | 50.03 |
| | | | <hr/> 50.03 |
| Jackson and Gillis method No. IV | | | |
| S. Byall | 49.72 | -16.67 | 50.06 |
| C. A. Gamble, J. E. Mull | 49.70 | -16.55 | 49.95 |
| | | | <hr/> 50.01 |
| <i>Sucrose plus aspartic acid.</i> | | | |
| Invertase method | | | |
| S. Byall | 49.68 | -16.29 | 49.94 |
| C. A. Gamble, J. E. Mull | 49.68 | -16.28 | 49.93 |
| | | | <hr/> 49.94 |
| A. O. A. C. acid method | | | |
| S. Byall | 49.69 | -16.42 | 49.63 |
| C. A. Gamble, J. E. Mull | 49.68 | -16.15 | 49.42 |
| | | | <hr/> 49.53 |
| Jackson and Gillis method No. II | | | |
| S. Byall | 49.63 | -16.94 | 49.93 |
| C. A. Gamble, J. E. Mull | 49.58 | -17.05 | 49.97 |
| | | | <hr/> 49.95 |
| Jackson and Gillis method No. IV | | | |
| S. Byall | 49.52 | -16.42 | 49.72 |
| C. A. Gamble, J. E. Mull | 49.53 | -16.15 | 49.52 |
| | | | <hr/> 49.62 |
| <i>Sucrose plus asparagine.</i> | | | |
| Invertase method | | | |
| S. Byall | 49.75 | -16.26 | 49.97 |
| C. A. Gamble, J. E. Mull | 49.88 | -16.20 | 50.02 |
| | | | <hr/> 50.00 |
| A. O. A. C. acid method | | | |
| S. Byall | 49.80 | -16.38 | 49.69 |
| C. A. Gamble, J. E. Mull | 49.88 | -16.08 | 49.52 |
| | | | <hr/> 49.61 |
| Jackson and Gillis method No. II | | | |
| S. Byall | 49.66 | -17.06 | 50.04 |
| C. A. Gamble, J. E. Mull | 49.68 | -16.90 | 49.93 |
| | | | <hr/> 49.99 |
| Jackson and Gillis method No. IV | | | |
| S. Byall | 49.61 | -16.38 | 49.76 |
| C. A. Gamble, J. E. Mull | 49.60 | -16.08 | 49.52 |
| | | | <hr/> 49.64 |

TABLE 2.
Invert sugar.

| ANALYST, AND METHOD USED | DIRECT POLARIZATION | INVERT POLARIZATION | PERCENTAGE |
|---|------------------------|------------------------|------------|
| Invertase method | | | |
| S. Byall | -16 91 | -16 93 | 0 03 |
| C. A. Gamble, J. E. Mull | -16 68 | -16.68 | 0.00 |
| | | | 0 02 |
| A. O. A. C. acid method | | | |
| S. Byall | -16 92 | -17 39 | 0 71 |
| C. A. Gamble, J. E. Mull | -16 68 | -17 15 | 0 71 |
| | | | 0.71 |
| Jackson and Gillis method No. II | | | |
| S. Byall | -17 76 | -17.66 | -0 15 |
| C. A. Gamble, J. E. Mull | -17.57 | -17.55 | -0.03 |
| | | | -0.09 |
| Jackson and Gillis method No. IV | | | |
| S. Byall | -17 49 | -17 39 | -0 15 |
| C. A. Gamble, J. E. Mull | -17.22 | -17.15 | -0.11 |
| | | | -0.13 |
| <i>Invert sugar plus aspartic acid.</i> | | | |
| Invertase method | | | |
| S. Byall | -17.21 | -17 32 | 0 17 |
| C. A. Gamble, J. E. Mull | -17 00 | -16 83 | -0.26 |
| | | | -0.04 |
| A. O. A. C. acid method | | | |
| S. Byall | -17 21 | -17 12 | -0.14 |
| C. A. Gamble, J. E. Mull | -17 00 | -16.80 | -0 30 |
| | | | -0.22 |
| Jackson and Gillis method No. II | | | |
| S. Byall | -18 00 | -17 37 | -0 94 |
| C. A. Gamble, J. E. Mull | -17.70 | -17 68 | -0 03 |
| | | | -0 49 |
| Jackson and Gillis method No. IV | | | |
| S. Byall | -17 72 | -17 12 | -0.90 |
| C. A. Gamble, J. E. Mull | -17.43 | -16 80 | -0.95 |
| | | | -0 93 |
| <i>Invert sugar plus asparagine.</i> | | | |
| Invertase method | | | |
| S. Byall | -16.82 | -16.82 | 0.00 |
| C. A. Gamble, J. E. Mull | -17 00 | -16 78 | -0 33 |
| | | | -0 17 |
| A. O. A. C. acid method | | | |
| S. Byall | -16.82 | -16 80 | -0 03 |
| C. A. Gamble, J. E. Mull | -17.00 | -16.70 | -0 45 |
| | | | -0.24 |
| Jackson and Gillis method No. II | | | |
| S. Byall | -17.58 | -17 35 | -0 34 |
| C. A. Gamble, J. E. Mull | -17.68 | -17.50 | -0 27 |
| | | | -0 31 |
| Jackson and Gillis method No. IV | | | |
| S. Byall | -17 37 | -16 80 | -0 86 |
| C. A. Gamble, J. E. Mull | -17 45 | -16 70 | -1 13 |
| | | | -1.00 |

Before starting the analyses it was necessary to ascertain whether the dextrose and levulose were suitable for this work; that is, whether they were free from sucrose and other compounds hydrolyzable by hydrochloric acid. Half-normal weights of the sugars were placed in 100 ml. flasks and dissolved in water, 10 cc. of the dilute hydrochloric acid used for inversion was added, and then the volume was completed to the mark. A reading was taken at 20° after a few minutes, and another reading after 24 hours' standing. With both the dextrose and the levulose there was no change in rotation, within the limits of experimental error.

The results of the collaborative analyses are shown in Tables 1, 2, and 3, for sucrose, invert sugar, and the mixture of the two. The tables are again arranged in the same manner as was done in previous reports.

TABLE 3.

Mixture of sucrose and invert sugar.

| ANALYST, AND METHOD USED | DIRECT POLARIZATION | INVERT POLARIZATION | SUCROSE FOUND | SUCROSE CALCULATED ON BASIS OF SAME METHOD |
|----------------------------------|------------------------|------------------------|------------------|---|
| Invertase method | | | | |
| C. A. Gamble, J. E. Mull | 16.60 | -16.38 | 49.93 | 49.98 |
| A. O. A. C. acid method | | | | |
| C. A. Gamble, J. E. Mull | 16.60 | -16.85 | 50.23 | 50.29 |
| Jackson and Gillis method No. II | | | | |
| C. A. Gamble, J. E. Mull | 16.05 | -17.25 | 49.95 | 50.01 |
| Jackson and Gillis method No. IV | | | | |
| C. A. Gamble, J. E. Mull | 16.18 | -16.85 | 49.80 | 49.90 |

Mixture of sucrose and invert sugar, plus aspartic acid.

| | | | | |
|----------------------------------|-------|--------|-------|-------|
| Invertase method | | | | |
| C. A. Gamble, J. E. Mull | 16.30 | -16.60 | 49.81 | 49.80 |
| A. O. A. C. acid method | | | | |
| C. A. Gamble, J. E. Mull | 16.30 | -16.50 | 49.25 | 49.27 |
| Jackson and Gillis method No. II | | | | |
| C. A. Gamble, J. E. Mull | 15.90 | -17.33 | 49.85 | 49.96 |
| Jackson and Gillis method No. IV | | | | |
| C. A. Gamble, J. E. Mull | 16.00 | -16.50 | 49.01 | 49.05 |

Mixture of sucrose and invert sugar, plus asparagine.

| | | | | |
|----------------------------------|-------|--------|-------|-------|
| Invertase method | | | | |
| C. A. Gamble, J. E. Mull | 16.43 | -16.48 | 49.83 | 49.86 |
| A. O. A. C. acid method | | | | |
| C. A. Gamble, J. E. Mull | 16.43 | -16.43 | 49.34 | 49.30 |
| Jackson and Gillis method No. II | | | | |
| C. A. Gamble, J. E. Mull | 15.95 | -17.25 | 49.80 | 49.80 |
| Jackson and Gillis method No. IV | | | | |
| C. A. Gamble, J. E. Mull | 16.10 | -16.43 | 49.05 | 48.96 |

Like last year, there were in some cases appreciable differences between the results obtained in the two laboratories. Since the work was done at each place at least in duplicate and with great care, there must be some reason for these discrepancies other than experimental error, and a possible explanation is offered below. Sam Byall did not have sufficient of the original dextrose and levulose to complete the work, and half of his analyses of the mixtures containing sucrose and invert sugar were made with a different lot of materials. The results thus obtained did not agree at all with those calculated from the figures for sucrose and for invert sugar alone, and for this reason only the analyses made at New York are reported in Table 3.

The average results obtained with sucrose (Table 1), alone or with aspartic acid or asparagine, check quite well with those reported last year, although Sam Byall's invert readings for the A. O. A. C. acid method and for Jackson and Gillis method No. IV are considerably higher than the corresponding figures obtained last year, when the agreement between the two laboratories was better. It is found again that in the case of sucrose mixed with amino compounds the A. O. A. C. acid method and Jackson and Gillis method No. IV yield too low results for sucrose. The reason for this was explained in last year's report. The invertase method and Jackson and Gillis method No. II gave correct sucrose figures in such mixtures.

With invert sugar alone (Table 2, first section) the invertase method and Jackson and Gillis methods No. II and IV would be expected to show zero sucrose. The analyses give practically zero for the invertase method. In the A. O. A. C. acid method the invert reading should be numerically 0.55, that is 0.5 (133.2–132.1), higher than the direct reading, and the apparent sucrose content should be 0.83 per cent. The result actually found, 0.71 per cent, is 0.12 lower than the theoretical. In the other two methods, where acid is used for the hydrolysis, Jackson and Gillis No. II and IV, the results are also slightly lower than the expected zero result, namely -0.09 and -0.13 per cent. This apparent negative sucrose figure, of 0.11 per cent on the average, is very probably due to a slight destruction of invert sugar at the temperature of 26° during the standing overnight, which destruction had not been noticeable in the preliminary tests made at a lower temperature. According to Jackson and Gillis¹ the decrease in the levorotation of invert sugar prepared from the normal weight of sucrose, in the presence of 0.7925 normal hydrochloric acid, is 0.056° V. per minute at 70° , or 0.028° for the half-normal weight. It is found by extrapolation of Jackson and Gillis' table that at 26° the decrease for the half-normal weight would be 0.000084° V. per minute, or 0.12° in 24 hours, or 0.08° in 16 hours. In

¹ Bur. Standards Sci. Paper 375, 142-3.

the presence of 0.634 normal hydrochloric acid, used ordinarily, it should be somewhat less. The decrease of 0.07° actually observed, and corresponding to 0.11 per cent apparent sucrose, is therefore of the expected order of magnitude. It should be stated here that this slight negative error does not affect the analysis of pure sucrose, because in that case it is taken care of by the experimentally determined Clerget divisor.

Summing up, with invert sugar alone, a sucrose determination, made under the experimental conditions used in this investigation, yields zero sucrose by the invertase method, a considerable quantity (about 0.7 per cent) of apparent sucrose by the A. O. A. C. acid method, and a small amount (about 0.1 per cent) of apparent negative sucrose by Jackson and Gillis methods No. II and IV.

When invert sugar is analyzed for sucrose in the presence of aspartic acid or asparagine, entirely different results are obtained. It is noted in the first place that, exactly like last year, the differences between the results of the two laboratories are in most instances very much greater than in the case of invert sugar alone. It is a well-known fact that amino acids and their amids react with carbohydrates in acid, neutral, or alkaline solution. Samuely¹ reported the formation of humin substances upon heating amino acids with inorganic acids in the presence of carbohydrates. Maillard² investigated the reaction between sugars and amino acids in aqueous solution, while Stanek³ worked in a slightly alkaline medium. More recently the reaction has been studied by Ripp⁴ and by Ambler⁵. It is quite plausible that the discrepancies in the results obtained by the two laboratories may be explained by unavoidable variations in the details of the procedure, especially as regards time and temperature.

This reaction readily accounts not only for the poor agreement in the results, but also for their tendency towards apparent negative sucrose. Both the invertase method and Jackson and Gillis method No. II should in the absence of reversion products yield zero sucrose, but they actually give slightly negative figures. The 0.94 per cent reported by Sam Byall for invert sugar and aspartic acid is probably due to experimental error.

According to last year's findings, the quantity of amino compounds present is shown by the differences between the results of Jackson and Gillis method No. II and No. IV: it was found to be 0.75 per cent, in terms of apparent sucrose. This year, when the same quantities of aspartic acid and asparagine were used, the average difference for the mixture of invert sugar and asparagine was found to be 0.69 per cent,

¹ *Centr.*, 805 (1902).

² *Compt. rend.*, 153, 1078 (1911); 154, 66 (1912).

³ *Z. Zuckerind. Böhmen.*, 41, 607 (1917).

⁴ *Z. Ver. deut. Zucker-Ind.*, 76: 627 (1926).

⁵ *Ind. Eng. Chem.*, 21, 47 (1929).

in fair agreement with last year's result. In the mixture of invert sugar and aspartic acid the average difference is only 0.43, due to the abnormally high negative result obtained by Sam Byall with Jackson and Gillis method No. II.

The results of the plain acid method should, according to previous findings, be 0.71 too high on account of the effect of the free hydrochloric acid on the rotation of the levulose, but 0.75 too low on account of the high dextrorotation of aspartic acid and of asparagine in acid solution. The sum total effect should be about zero sucrose. Actually, a small percentage of apparent negative sucrose is found, evidently due to the destruction of invert sugar by interaction with the amino compounds, which reaction affects all methods in about the same manner.

In Table 3 fair agreement is noted between the results found and the corresponding figures calculated for the mixtures from those obtained for the components (Tables 1 and 2), on the basis of the same method. This way of judging the results affords only a relative measure of their correctness, because it takes into consideration the inaccuracies of the acid hydrolysis methods when applied to invert sugar with or without amino compounds.

From the standpoint of absolute correctness, there is, as was to be expected, practically no difference between the results of the invertase method and Jackson and Gillis method No. II, no matter whether amino compounds are present or not. This is, of course, due to the absence of reversion products.

In the absence of amino compounds, Jackson and Gillis method No. IV gives the same results as Jackson and Gillis method No. II, again in accordance with previous findings. On the other hand, when amino compounds are present, the result of Jackson and Gillis method No. II differs from that of No. IV by 0.76 in the aspartic acid series, and by 0.71 in the asparagine series, compared to 0.75 found last year under the same conditions. The conclusion reached last year, that the difference between the results of Jackson and Gillis methods No. II and IV gives an approximate measure of the amino compounds present, has thus been confirmed.

Previous results had indicated that the difference between the results of the invertase method and of Jackson and Gillis method No. II gives an approximate measure of the reversion products. This implies that in the absence of reversion products the two methods should give identical results, and zero sucrose in the absence of sucrose, no matter whether amino compounds are present or not. This year's work goes to show that this is strictly true only for pure sucrose, with or without amino compounds. With invert sugar alone, the invertase method actually gives zero sucrose, but Jackson and Gillis method No. II is liable to give slightly negative results, owing to the destructive effect

of hydrochloric acid on invert sugar even at room temperatures prevalent during the summer months. When aspartic acid or asparagine is also present with the invert sugar, then not only Jackson and Gillis method No. II, but even the invertase method may not give exactly zero sucrose, but both tend to indicate small quantities of apparent negative sucrose. This is most likely caused by the reaction between invert sugar and amino compounds in the course of the analysis itself. It remains to be seen whether this difficulty can be overcome by inverting at lower temperatures during a longer time.

The observations made this year require further study and confirmation. They are of no great importance from the standpoint of practical sugar analysis, because they deal with extreme cases. With actual cane products containing sucrose, invert sugar, reversion products, and amino compounds, the effect of the reaction between invert sugar and amino compounds would be only slight and of no great consequence for the determination of sucrose.

As this entire investigation has now been carried to a certain point where it cannot be continued without further fundamental research, there is presented below a summary of the conclusions reached by the associate referee during the last six years concerning the polariscopic determination of sucrose in mixtures characterized in the paragraph immediately above.

SUMMARY.

(1) The solution used for the direct polarization must have the same dry substance concentration as that used for the invert polarization.

(2) The Clerget divisor to be used must be based on the total sugar (or dry substance) concentration, and not on the difference between the direct and the invert polarization.

(3) Of the four inversion methods investigated, viz., invertase method, A. O. A. C. acid method, and Jackson and Gillis methods No. II and No. IV, with the inversion carried out at an average room temperature of about 26°, the invertase method is the only one which can be depended upon to yield the exact (in the absence of amino compounds) or very nearly exact (in the presence of amino compounds) percentage of sucrose present.

(4) The sucrose result by Jackson and Gillis method No. II is increased by reversion products hydrolyzed under the conditions of the experiment, but it is only slightly affected by the presence of amino compounds.

(5) The sucrose result by Jackson and Gillis method No. IV is increased by the hydrolysis of reversion products in the same way as that by method No. II, but it is appreciably lowered in the presence of asparagine or aspartic acid.

(6) The difference between the sucrose result by Jackson and Gillis method No. II and that by the invertase method gives an approximate measure of the reversion products hydrolyzed by hydrochloric acid under the conditions of the analysis.

(7) The difference between the sucrose result by Jackson and Gillis method No. II and that by No. IV gives an approximate measure of the asparagine or aspartic acid present.

(8) The plain acid method may give any kind of a result, depending on the relative proportions between the different constituents of the mixture analyzed.

(9) It is preferable to carry out the inversion at room temperature, because at high temperatures slight variations in the time used may have an appreciable effect on such reactions as the destruction of invert sugar in the presence of strong acid, on the hydrolysis of reversion products, and on the interaction between invert sugar and amino compounds.

RECOMMENDATIONS.

In view of the results obtained this year, it is recommended—

(1) That a fundamental study be made of the effect of the simultaneous presence of invert sugar and of amino compounds on the determination of sucrose by inversion methods. This should preferably be done in the form of an individual research project rather than a collaborative investigation. It may later be followed by such collaborative studies as the results may warrant.

Work on recommendation No. 3, presented in the report for 1927, was suspended during the past two years in order to permit completion of more important investigations. It is now recommended that it be taken up actively again and

(2) That the associate referee for next year study the effect of lead clarification on the results of Clerget determinations in cane sugar products.

REPORT ON CHEMICAL METHODS FOR REDUCING SUGARS.

By R. F. JACKSON (Bureau of Standards, Washington, D. C.),
Associate Referee.

The gravimetric method for the determination of reducing sugars which is most commonly used at the present time is the unified method of Munson and Walker¹. It was announced as a unified method because the same procedure was specified for all reducing sugars. The authors determined the copper-sugar equivalents for a number of the

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 59 (1930).

² *J. Am. Chem. Soc.*, 28, 663 (1906); 29, 652 (1907); *Methods of Analysis*, A. O. A. C., 1925, 190.

TABLE 1.
Copper-reducing equivalents of dextrose and levulose by Munson and Walker's method.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|-----------------------------|-----------------|--|-----------------------------|-----------------|--|---|---------------------|--|--|---|
| WEIGHT DEXTROSE TAKEN | COPPER FOUND | COPPER FROM MUNSON AND WALKER'S TABLE | WEIGHT LEVULOSE TAKEN | COPPER FOUND | WEIGHT LEVULOSE YIELDING SAME COPPER AS DEXTROSE | RATIO OF WEIGHTS OF LEVULOSE TO DEXTROSE OBSERVED | RATIO FROM CURVE | MUNSON AND WALKER'S TABLE EXTRAPOLATED TO LEVULOSE | RATIO: WEIGHTS OF INVERT SUGAR TO DEXTROSE (MUNSON AND WALKER) | REDUCING POWER OF LEVULOSE (RECIPROCAL OF COL. 8) |
| mg. 200 | mg. 375.1 | mg. 374.7 | mg. 213.1 | mg. 374.9 | mg. 213.1 | 1.066 | 1.067 | 1.056 | 1.028 | 0.937 |
| 200 | 375.9 | 374.7 | 212.5 | 374.1 | 213.5 | 1.068 | 1.067 | 1.056 | 1.028 | 0.937 |
| 160 | 307.0 | 306.8 | 170.5 | 303.1 | 172.7 | 1.079 | 1.075 | 1.060 | 1.030 | 0.930 |
| 120 | 234.6 | 235.4 | 127.9 | 231.0 | 129.8 | 1.082 | 1.082 | 1.065 | 1.033 | 0.924 |
| 100 | 197.7 | 198.3 | 106.6 | 194.4 | 108.4 | 1.084 | 1.086 | 1.067 | 1.034 | 0.921 |
| 85.75 | 173.0 | 171.5 | 94.03 | 173.6 | 93.7 | 1.093 | 1.089 | 1.070 | 1.035 | 0.918 |
| 64.31 | 129.9 | 130.2 | 70.52 | 130.8 | 70.0 | 1.089 | 1.093 | 1.074 | 1.037 | 0.915 |
| 42.88 | 88.8 | 87.9 | 47.01 | 88.8 | 47.0 | 1.097 | 1.097 | 1.079 | 1.040 | 0.912 |

more important sugars and sugar mixtures, but probably on account of its rarity they omitted the data for levulose. The associate referee has in the past year made some preliminary experiments for the purpose of determining these equivalents.

The details of the analyses were carried out rigorously in accordance with Munson and Walker's specifications, except that copper was determined by thiosulfate titration instead of by direct weighing of the copper precipitate. The titration method is less tedious and eliminates many of the uncertainties inherent in gravimetric analysis.

Immediately preceding the analysis of each levulose solution a similar analysis was conducted with a standard dextrose solution of such sugar content that both precipitated approximately the same weight of copper.

In Table 1 are shown the data obtained. Columns 2 and 3 show that the present experiments are in essential agreement with Munson and Walker's data on dextrose. Column 6 shows the weight of levulose (calculated from columns 4 and 5) which yields the same weight of copper (column 2) as the dextrose in column 1, and column 7 the ratio of weights of the sugars. These observed ratios were plotted against the weight of dextrose and from the smoothed curve the values in column 8 were read. The reciprocals of these values are given in column 11.

From column 8 it is apparent that the reducing ratio is a function of the concentration of sugar. Evidently the practise of employing a single-valued ratio, regardless of concentration of sugar, is hazardous, unless it has been demonstrated that the ratio is constant.

In column 10 are the ratios of weights of invert sugar to dextrose computed from Munson and Walker's table. If it is assumed that the reducing power of a sugar mixture is an additive property of the constituents, the ratios of invert sugar to dextrose can be extrapolated to those of levulose to dextrose, as is done in column 9. A comparison of these extrapolated with the experimental ratios (column 8) reveals a serious discrepancy. Either the rule of mixtures is inaccurate or some error exists in the experimental data. Such error may occur either in the present data for levulose or in Munson and Walker's values for invert sugar.

In seeking the source of this discrepancy it is the purpose of the associate referee to conduct further analyses of pure levulose. At the present time it is of interest to study these respective ratios as determined by other workers and by different methods. Table 2 shows the results of this search. Meissl's ratios for invert sugar are directly comparable with those of Munson and Walker since he used the same solutions and the same period of boiling, and yet he obtained a value nearly 2 per cent higher. In general the results in Table 2 indicate that invert sugar is approximately intermediate in reducing power between dextrose and levulose, justifying in some degree the extrapolation of Munson and

Walker's value for invert sugar to levulose. In every instance the ratio of invert sugar to dextrose is higher than Munson and Walker's value. The author's values for levulose are in closer agreement with those of other workers than with the values extrapolated from Munson and Walker's invert sugar-dextrose ratio.

TABLE 2.
Ratios of reducing equivalents by various methods.

| INVESTIGATORS | 200 MG. DEXTROSE = 1.000 | | 100 MG. DEXTROSE = 1.000 | |
|--------------------------------------|------------------------------------|--------------------------------|------------------------------------|--------------------------------|
| | Ratio: Invert sugar Dextrose | Ratio: Levulose Dextrose | Ratio: Invert sugar Dextrose | Ratio: Levulose Dextrose |
| Munson and Walker..... | 1.028 | (1.056)* | 1.034 | (1.068)* |
| Meissl..... | 1.045 | | 1.054 | |
| Brown, Morris, and Millar..... | 1.033 | 1.070 | 1.052 | 1.098 |
| Kjeldahl and Woy (50 cc. Fehling)... | 1.037 | 1.072 | 1.049 | 1.094 |
| Kjeldahl and Woy (30 cc. Fehling)... | | | 1.047 | 1.088 |
| C. A. Browne (Allihn's Solution)... | | | 1.044 | 1.093 |
| Quisumbing and Thomas..... | 1.039 | 1.074 | 1.050 | 1.091 |
| Jackson..... | | 1.067 | | 1.086 |

* Extrapolated.

The results of these experiments on levulose and of the survey show the need of reconciling the discordant data on the reducing powers of levulose and of invert sugar.

For the volumetric method of Lane and Eynon¹ a high degree of precision was claimed by the authors. Since the publication of the original article no confirmatory expression has been made by other workers. It seemed desirable to do this and to investigate the effect of variation in the time required for titration since the latter is necessarily variable. In Table 3 are given two typical series of titrations out of many which were performed. The data show not only that the attainable precision is high but that relatively large variations in time of titration produce but little effect on the result. Enclosed in brackets are those titrations which conform to the specifications of the method.

The selective determination of levulose by Nyns' method has been the subject of study for a number of years. In the original specifications Nyns² stated that 60 mg. of levulose was the maximum quantity of sugar which could be analyzed. The author, on the other hand, has found that more than 90 mg. can be determined. F. W. Zerban has called to the author's attention that the discrepancy arose from a typographical error in *Chemical Abstracts*³. It seems that the typosetters have made a considerable contribution to the progress of the method, since in the associate referee's experience the higher concentrations of levulose yield the more reliable and reproducible results.

¹ *J. Soc. Chem. Ind.*, 32T (1923).

² *Bull. assoc. école sup. brasserie Louvain*, 25, 63 (1925); *C. A.*, 19, 1236 (1925).

³ *C. A.*, 19, 1236 (1925).

TABLE 3.

Effect of time on Lane and Eynon titration.

| LEVULOSE | | | 80% LEVULOSE 20% DEXTROSE | | |
|----------|---------|-----------|------------------------------|---------|-----------|
| Time | | Titration | Time | | Titration |
| minutes | seconds | | minutes | seconds | |
| 2 | 30 | 25.22)* | 1 | 21 | 21 26 |
| 2 | 35 | 25 24} | 1 | 30 | 21 20 |
| 2 | 59 | 25.23} | 1 | 55 | 21 20 |
| 3 | 5 | 25 24} | 2 | 10 | 21 11 |
| 3 | 44 | 25.20 | 2 | 20 | 21 20)* |
| 3 | 46 | 25 20 | 2 | 53 | 21 18} |
| 4 | 52 | 25 20 | 3 | 9 | 21 15} |

* Titrations that conform to the method.

RECOMMENDATIONS¹.

It is recommended—

(1) That the reducing power of invert sugar by Munson and Walker's method be studied with a view to corroborating or revising Munson and Walker's tables, and that further experiments be made to determine the reducing power of levulose.

(2) That the study of Nyns' selective method for the determination of levulose be continued, and that the effect of aldohexoses and pentoses be determined.

(3) That the iodine method for the determination of aldose sugars be studied, with particular reference to the modification devised by Slater and Acree².

COMMITTEES NAMED BY THE PRESIDENT.

Committee to Wait upon Secretary of Agriculture: R. N. Brackett and J. W. Kellogg.

Committee to Wait upon Honorary President: B. B. Ross, H. J. Patterson and G. S. Fraps.

Committee on Resolutions: W. W. Randall and H. C. Lythgoe.

Committee on Auditing: A. E. Paul and L. E. Bopst.

Committee on Nominations: C. D. Howard, W. H. MacIntire, and H. A. Lepper.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 59 (1930)

² Unpublished manuscript.

FIRST DAY.

MONDAY—AFTERNOON SESSION.

REPORT ON FERTILIZERS.

By G. S. FRAPS (Agricultural Experiment Station, College Station, Tex.),
Referee.

The work on fertilizers made satisfactory progress, as is shown by the reports and recommendations of the associate referees.

The following resolution was adopted by the Fertilizer Division at the Minneapolis meeting of the American Chemical Society, September 10-11, 1929:

RESOLVED: That in view of the present trend in fertilizer practice toward mixtures of higher analysis and the use therein of more concentrated fertilizer materials, extreme care is required in the sampling, preparation of samples for analysis, and the analysis of such samples.

And be it further

RESOLVED: That the Fertilizer Referee of the A. O. A. C. be requested to have carefully studied these important matters, including among others finer grinding of the sample in preparation for analysis; more accurate determination of all ingredients, with special attention to the determination of total nitrogen, including digestion of the sample and especially in distillation of the ammonia and its absorption in standard acid to avoid loss of NH_3 . *And be it further*

RESOLVED: That the referee be requested to undertake an intensive study of the methods for the determination of the various forms of nitrogen.

The referee is inclined to believe that the analysis of high analysis fertilizer should receive some additional attention, and therefore recommends the appointment of an Associate Referee on Methods for High Analysis Fertilizers for the purpose of studying special methods of preparation or analysis required by such fertilizers. Some of these fertilizers are hygroscopic or present other difficulties, and as they promise to become of increasing importance, it seems proper to devote special attention to them. The other associate referees already have sufficient work, and it seems better, at present, to have an associate referee to examine the problem, see what is needed to be done, if anything, and arrange for such work as may be necessary either alone or in cooperation with the other associate referees.

The referee had occasion to review the work done on methods for the estimation of nitrogen, in connection with the revision of the chapter on nitrogen in Dr. Wiley's book. It seems, from the literature, that the Kjeldahl-Gunning method, using both potassium or sodium sulfate and mercury, is much superior to the other methods, with some materials in accuracy and in other materials in speed of digestion. The referee is

inclined to recommend that the other methods for mixed fertilizers be dropped, and that the salicylic acid-mercury-potassium sulfate method be retained for mixed fertilizers containing nitrates. However, this proposal must be discussed thoroughly before any recommendation is made.

Some blank tests made by A. J. Sterges in the laboratory of the referee on different types of bulbs used in the zinc-ferrous-sulfate method are of interest in this connection. The averages of several sets of three blank tests each were as follows: ordinary bulb, Eimer and Amend No. 28902, 0.97, 0.63 and 0.82 cc. 0.2 *N* acid; Clark bulb, Eimer and Amend No. 28916, 0.17, 0.22 and 0.26 cc.; McHargue bulb, Eimer and Amend No. 28920, 0.35, 0.39 and 0.41 cc. The blank with the ordinary type bulb is high and irregular. The other two types give much better results. While the difference between them is small, yet the Clark bulb is superior. It may be advisable to specify more exactly the kind of distillation bulb to be used in some of the methods.

RECOMMENDATION¹.

The referee recommends that an associate referee be appointed for work on methods of preparation or analysis of high analysis fertilizers.

THE VOLUMETRIC DETERMINATION OF PHOSPHORIC ACID.

By WM. H. ROSS (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

At last year's meeting of this association a report² was given on the influence of sulfates on the determination of phosphoric acid by the volumetric method. It was found that the presence of sulfates gave high results in this determination when the phosphoric acid was precipitated at 45°–50°C., as described under paragraph 10 (a), *Methods of Analysis*, but that the interference of sulfates was slight when the precipitation was made at room temperature (25°–30°C.), with continuous stirring, as described under paragraph 10 (b). It was accordingly recommended (1) that the work be repeated this year on a quantity of sulfate approximating that usually present when a superphosphate of ordinary grade is analyzed, and (2) that a study be made of the possibility of using sulfuric acid, as described under paragraph 6 (d), *Methods of Analysis*, in the preparation of solutions of samples high in organic matter for analysis by the volumetric method.

Three standard phosphate samples were selected for this work. Sam-

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 60 (1930).

² *This Journal*, 12, 170 (1929).

ple No. 1 was pure monopotassium phosphate prepared by the method described in last year's report; Sample No. 2 was the Tennessee phosphate rock standard sample No. 56 of the Bureau of Standards; and Sample No. 3 was the portion of a commercial grade of cottonseed meal that passed through a 100-mesh screen.

The directions sent to the collaborators called for—

(A) the volumetric determination of phosphoric acid in sample No. 1 with and without sulfate added by (1) precipitating the phosphoric acid at 45°–50°C., as directed under paragraph 10 (a), and (2) precipitating at 25°–30°C. with continuous stirring, as directed under paragraph 10 (b); (B) the determination of phosphoric acid in a mixture of equal parts of sample No. 2 and sample No. 3 by (1) the gravimetric method, and (2) the volumetric method of precipitating at 25°–30°C. with continuous stirring; and (C) the gravimetric determination of phosphoric acid in sample No. 3.

DIRECTIONS FOR COLLABORATIVE WORK.

A. Determine total P_2O_5 in Sample No. 1 by the following procedures, using the reagents listed in the last edition of Methods of Analysis, A. O. A. C., p. 3, sec. 8.

(1) Dry sample at 105°C. for 1 hour. Dissolve 2.5 grams of the sample in water and dilute to 500 cc. Withdraw an aliquot of 10 cc., add 10 grams of ammonium nitrate, acidify with a few drops of nitric acid, dilute to 75–100 cc., heat in a water bath to 45°–50°C., and add 35 cc. of the molybdate solution. Allow the mixture to remain in the bath, stirring occasionally, for 30 minutes; decant at once through a filter, wash the precipitate twice by decantation with 25–30 cc. portions of water, agitating thoroughly, and allow to settle; then transfer the precipitate to the filter and wash it with cold water until the filtrate from two fillings of the filter (9 cm.) yields a pink color upon the addition of phenolphthalein and one drop of the standard alkali. Transfer the precipitate and filter to the beaker or precipitating vessel, dissolve the precipitate in a small excess of the standard alkali, add about six drops of phenolphthalein indicator, and titrate with the standard acid (official volumetric method 10 (a)).

(2) Withdraw a second 10 cc. aliquot of the solution prepared as directed under A (1), add 0.15 gram of potassium sulfate and 10 grams of ammonium nitrate, and complete the determination as directed under A (1).

(3) Withdraw a third 10 cc. aliquot of the solution prepared as directed under A (1), add 10 grams of ammonium nitrate, acidify with a few drops of nitric acid, and dilute to 75–100 cc. Adjust the temperature of the solution, if necessary, to 25°–30°C., place in a stirring apparatus, add 35 cc. of the molybdate solution, stir for at least 30 minutes at room temperature, filter, wash, and complete the determination as directed under A (1) (official volumetric method 10 (b)).

(4) Withdraw a fourth 10 cc. aliquot of the solution prepared as directed under A (1), add 0.15 gram of potassium sulfate and 10 grams of ammonium nitrate, and complete the determination as directed under A (3).

B. Determine total P_2O_5 in a mixture of equal parts of Sample No. 2 and Sample No. 3 as follows:

(1) Dry Sample No. 2 at 105°C. for 1 hour. Weigh 1 gram of the dry sample and 1 gram of Sample No. 3 without previous drying into a 200 cc. flask. Add 5 cc. of concentrated nitric acid and 20–30 cc. of concentrated sulfuric acid and allow to digest, at a gentle heat if necessary, until the violence of the reaction is over. Boil and add 2–4 grams of sodium nitrate, or potassium nitrate, at the beginning of the digestion, and a small quantity after the solution has become nearly colorless, or add the nitrate

TABLE 1.
Analysis of standard phosphate samples.

| COLLABORATORS | SAMPLE NO. 1* VOLUMETRIC METHOD | | | | EQUAL PARTS OF SAMPLE NO. 2 AND NO. 3† | | SAMPLE NO. 3 |
|-------------------------------|-------------------------------------|----------------------------|--------------------------------------|----------------------------|---|---|--------------|
| | Sulfates absent Precipitation at | | Sulfates present Precipitation at | | Gravimetric method | Volumetric method Sulfates present Precipitation at 25°-30°C. with stirring | |
| | 25°-30°C. with stirring | | 25°-30°C. with stirring | | | | |
| | 45°-50°C. | 25°-30°C. with stirring | 45°-50°C. | 25°-30°C. with stirring | | | |
| H. R. Allen | per cent | per cent | per cent | per cent | per cent | per cent | per cent |
| Kentucky Agr. Expt. Sta. | 52.40 | 52.40 | 53.80 | 52.50 | 17.40 | 17.50 | 3.48 |
| J. G. Asher | 52.25 | 52.20 | 53.40 | 52.30 | 17.34 | 17.72 | 3.53 |
| Virginia-Carolina Chem. Corp. | 52.40 | 52.30 | 52.70 | 52.35 | 17.68 | 17.78 | 3.57 |
| W. R. Austin | 52.35 | 52.20 | 53.80 | 52.30 | 17.56 | 17.50 | 3.42 |
| Armour Fertilizer Works | 52.88 | 52.50 | 55.36 | 52.70 | 17.48 | 17.48 | 3.51 |
| C. M. Bible | 52.30 | 52.20 | 53.00 | 52.40 | 17.56 | 17.58 | 3.58 |
| Mellon Institute | 53.08 | 51.76 | 55.20 | 52.24 | 17.51 | 17.60 | 3.45 |
| J. E. Breckenridge | 52.17 | 52.20 | 53.98 | 52.45 | 17.46 | 17.88 | 3.50 |
| Am. Agr. Chem. Co. | 52.33 | 52.21 | 52.93 | 52.21 | 17.40 | 17.31 | 3.52 |
| Armour Fertilizer Works | 52.07 | 52.48 | 53.41 | 52.49 | 17.44 | 17.48 | 3.47 |
| A. W. Clark | 52.20 | | 54.20 | | 17.47 | | 3.55 |
| New York Agr. Expt. Sta. | 52.15 | 52.00 | 54.05 | 52.05 | 17.39 | 17.45 | 3.51 |
| James B. Martin | 52.38 | 52.22 | 53.82 | 52.36 | 17.47 | 17.57 | 3.51 |
| Bur. Chemistry and Soils | .28 | .20 | .80 | .17 | .09 | .16 | .05 |
| D. S. Reynolds | | | | | | | |
| Bur. Chemistry and Soils | | | | | | | |
| Wm. A. Ryder | | | | | | | |
| F. S. Royster Guano Co. | | | | | | | |
| Boyd L. Samuel | | | | | | | |
| Dept. Agr. & Immigration, Va. | | | | | | | |
| Mean | | | | | | | |
| Standard deviation from Mean | | | | | | | |

* Phosphoric acid (P_2O_5) present—52.18 per cent.† Phosphoric acid (P_2O_5) present (assuming sample No. 3 contains 3.51 per cent of P_2O_5)—17.42 per cent.

in small portions from time to time. When the solution is nearly colorless, add 150 cc. of water and boil for a few minutes. Cool, dilute to the mark, mix, and pour on a dry filter or allow to settle. Withdraw an aliquot of 50 cc., add strong ammonium hydroxide in slight excess, and barely dissolve the precipitate formed with a few drops of strong nitric acid, stirring vigorously. Add about 15 grams of dry ammonium nitrate or a solution containing that quantity. To the hot solution add 70 cc. of the molybdate solution, digest at about 65°C. for 1 hour, and determine whether or not the phosphoric acid has been completely precipitated by the addition of more molybdate to the clear supernatant liquid. Filter and wash with cold water or preferably ammonium nitrate solution. Dissolve the precipitate on the filter with dilute ammonium hydroxide (1+1) and hot water and wash into a beaker to a volume of not more than 100 cc. Neutralize with strong hydrochloric acid, using litmus, or brom thymol blue, as indicator. Cool and from a buret add slowly (about one drop per second), stirring vigorously, 15 cc. of magnesia mixture. After 15 minutes add 12 cc. of strong ammonium hydroxide and allow the mixture to stand until the supernatant liquid is clear (usually 2 hours); filter, and wash the precipitate with the dilute ammonium hydroxide solution (2.5 per cent by weight) until the washings are practically free from chlorides; dry, burn first at a low heat, and ignite to constant weight, preferably in an electric furnace at 950°–1000°C. Cool in a desiccator and weigh as magnesium pyrophosphate ($Mg_2P_2O_7$). Calculate, and report the results as percentages of phosphoric acid (P_2O_5) in 2 grams of sample (official gravimetric method).

(2) Withdraw a 20 cc. aliquot of the solution prepared as directed under B (1). Add 10 grams of ammonium nitrate and then strong ammonia in slight excess, and barely dissolve the precipitate formed with a few drops of nitric acid (1 + 1), stirring vigorously. Dilute to 75–100 cc., adjust the temperature of the solution, if necessary, to 25°–30°C., place in a stirring apparatus, add 45 cc. of the molybdate solution, and stir continuously for at least 30 minutes at room temperature. Filter, wash, and complete the determination as directed under A (1) (official volumetric method, 10 (b)).

C. Determine total P_2O_5 in Sample No. 3.

(1) Weigh 2 grams of the sample, without previous drying, into a 200 cc. flask and proceed as directed under B (1) with the variation that the quantity of molybdate solution used should be decreased to about 35 cc., and the magnesia mixture to about 10 cc.

The results reported by the collaborators are given in Table 1.

DISCUSSION OF RESULTS.

Table 1 shows—

(1) That good results are obtained in the determination of phosphoric acid by both the official procedures of the volumetric method, when sulfates are absent, and that the agreement with the theoretical value is particularly close when the determination is made by precipitating at 25°–30°C. with continuous stirring.

(2) That the mean values reported by the collaborators this year for the phosphoric acid (P_2O_5) content of sample No. 1 agree almost exactly with those reported last year except when the precipitation was made at 45°–50°C. in the presence of sulfates.

(3) That the procedure of precipitating at 45°–50°C., as described under paragraph 10 (a), *Methods of Analysis*, A. O. A. C., gives high

results in the presence of sulfates even when the quantity present is no greater than that occurring in the ordinary grade of superphosphate.

(4) That the procedure of precipitating at 25°–30°C. with continuous stirring, as described under paragraph 10 (b), gives as good results in the presence of sulfates as are obtained with procedure 10 (a) in the absence of sulfates. It may be noted also that the standard deviation from the mean is less when the determinations are made with procedure 10 (b) than when made with procedure 10 (a).

The phosphoric acid (P_2O_5) in sample No. 2, that is the Bureau of Standards sample No. 56, is 31.33 per cent. If the mean value (3.51 per cent) reported by the collaborators for the phosphoric acid (P_2O_5) content of the cottonseed meal sample No. 3 be assumed to be correct, then the phosphoric acid (P_2O_5) in a mixture of equal parts of sample No. 2 and sample No. 3 should amount to 17.42 per cent. This value is in good agreement with the mean of the results reported by the collaborators for the phosphoric acid (P_2O_5) in a mixture of equal parts of sample No. 2 and sample No. 3 when determined both by the gravimetric method and by procedure 10 (b) of the volumetric method. The solution of the mixture for both determinations was prepared by dissolving in sulfuric acid as described under paragraph 6 (d).

It may be concluded, therefore, that the solution of the sample in sulfuric acid gives good results in the volumetric determination of phosphoric acid in samples high in organic matter providing the precipitation of the phosphoric acid is made at 25°–30°C. with continuous stirring.

RECOMMENDATIONS¹.

It is recommended—

(1) That the procedure of precipitating at 45°–50°C. as described under paragraph 10 (a), *Methods of Analysis*, A. O. A. C., be stated as not applicable to the volumetric determination of phosphoric acid in the presence of sulfates.

(2) That the sulfuric acid method for the preparation of solution, as described under paragraph 6 (d), *Methods of Analysis*, A. O. A. C., be adopted as an optional official method for use in the volumetric determination of phosphoric acid in samples high in organic matter.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 60 (1930).

REPORT ON NITROGEN.

By A. L. PRINCE (Agricultural Experiment Station,¹ New Brunswick, N. J.), *Associate Referee*.

It was recommended last year that the associate referee carry on further collaborative work comparing the Robertson² and Jones³ methods for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide or urea. In 1927 the Jones method was adopted tentatively because no other method met the situation so well. Shortly after this action was taken, however, the Robertson method was suggested as a simpler procedure for separating the various forms of nitrogen in a mixed fertilizer containing cyanamide and urea, with special reference to the determination of nitrate nitrogen. It appeared from the preliminary work⁴ carried on last year by the associate referee that this method was not only simpler than the Jones method, but that possibly it was more accurate, and therefore extensive collaborative work was planned to test out these findings.

A modification of the Robertson method suggested by C. H. Jones of the Vermont Agricultural Experiment Station was also tried out this year, but this modification may be used only when nitrate nitrogen is desired in the mixed fertilizers because it does not consider the water-soluble nitrogen. Inasmuch as such a situation often presents itself to the chemist, this shorter modification was given a try-out this year.

The two samples used for collaborative study were the same as those on which the preliminary work was done last year. They were prepared as follows:

| | PARTS PER 100 | | NITROGEN BY ANALYSIS <i>per cent</i> | NITROGEN IN SAMPLE— CALCULATED | |
|------------------------------|---------------|-------|---|-----------------------------------|--------------------------|
| | No. 1 | No. 2 | | No. 1 <i>per cent</i> | No. 2 <i>per cent</i> |
| Superphosphate | 60 | 60 | | | |
| Potassium chloride | 10 | 10 | | | |
| Sodium nitrate | 10 | 10 | 15.90 | 1.59 | 1.59 |
| Calcium cyanamide | 5 | 5 | 19.93 | 1.00 | 1.00 |
| Urea | 5 | | 46.39 | 2.32 | |
| Ammonium sulfate | 5 | 5 | 20.81 | 1.04 | 1.04 |
| Dried fish | 5 | 10 | 7.91 | 0.40 | 0.79 |
| | 100 | 100 | | 6.35 | 4.42 |

The cyanamide and urea used in sample No. 1 were equivalent to 100 pounds per ton of each substance; 100 pounds per ton of cyanamide was also used in sample No. 2. The samples were sent out to 20 chemists; 16 reports were received.

¹Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

²*This Journal*, 12, 177 (1929).

³*Ibid.*, 10, 198 (1927).

⁴*Ibid.*, 12, 176 (1929).

INSTRUCTIONS TO COLLABORATORS.

Experiments.

Series I.—Run each sample by the Robertson method as described below. Run in duplicate or triplicate. Under section 4, be sure to add the mixture of zinc dust and granular zinc (20-mesh) before distillation, or bumping will ensue.

Series II.—Run each sample by the Jones method as described below. Run in duplicate or in triplicate if time permits.

Series III.—Run each sample by the modified Robertson method as described below.

Report all results as percentage of nitrogen. Under the Robertson method it will be necessary to record and report the following forms of nitrogen: Total nitrogen, water-insoluble nitrogen, water-soluble nitrogen, nitrogen after ferrous sulfate treatment, nitrate nitrogen and ammonia nitrogen. Under the Jones method, record and report only the nitrate nitrogen and ammonia nitrogen.

ROBERTSON METHOD FOR DETERMINING NITRATE NITROGEN IN FERTILIZERS
CONTAINING CYANAMIDE, UREA, ETC.

1. Determine the total nitrogen by the usual methods modified to include nitrates.
2. Weigh out 2 grams of the fertilizer mixture, wash to 200 cc., and determine the nitrogen in the residue by any of the modifications of the Kjeldahl method. The difference between these two determinations gives the water-soluble nitrogen.

3. Distil 50 cc. of the filtrate, equivalent to 0.5 gram, with magnesium oxide for the determination of ammoniacal nitrogen, as described in *Methods of Analysis*, A. O. A. C., 1925, 11.

4. Take another 50 cc. portion of the same solution and put into a 500 cc. Kjeldahl flask, together with 2 grams of ferrous sulfate and 20 cc. of sulfuric acid, sp. gr. 1.84. Digest over a hot flame. After the water is evaporated and white fumes appear, continue the digestion for at least 10 minutes. The nitrate nitrogen is thereby driven off. (If the solution does not clear, add 0.65 gram of mercury, and boil until clear, then after dilution add potassium sulfide solution to precipitate mercury as usual.) Cool, dilute, and distil with strong caustic soda in the usual way. Add a pinch of a mixture of zinc dust and granular zinc (20-mesh) to each flask before distillation to prevent bumping.

1 (total) — 2 (water-insoluble) gives water-soluble nitrogen.

The difference between the water-soluble nitrogen and the nitrogen obtained in 4 gives the nitrate nitrogen.

3 (ammonia nitrogen) — nitrate nitrogen gives total mineral nitrogen.

JONES METHOD.

This method has been published¹.

MODIFIED ROBERTSON METHOD.

Weigh 0.5 gram of the sample into a Kjeldahl flask. Add 50 cc. of water and rotate gently. Add 2 grams of ferrous sulfate and rotate. Then add 20 cc. of sulfuric acid, sp. gr. 1.84, and digest over a hot flame. When the water is evaporated and white fumes appear, add 0.65 gram of mercury and complete the digestion as in the regular Kjeldahl method. Cool, dilute, and distil as usual. The nitrogen thus formed subtracted from the total nitrogen represents the nitrate nitrogen.

¹ *This Journal*, 10, 198 (1927).

TABLE 1.

Analysis of samples No. 1 and No. 2.

(Results expressed as percentage of nitrogen.)

| COLLABORATOR NO. | Robertson Method | | | | | | | | Jones Method | | | |
|-------------------|------------------|--------------------------|------------------------|--|------------------|---------------------|------------------------|------------------|------------------|---------------------|------------------------|------------------|
| | Total nitrogen | Water-insoluble nitrogen | Water-soluble nitrogen | Nitrogen after FeSO_4 treatment | Nitrate nitrogen | Ammoniacal nitrogen | Total mineral nitrogen | Organic nitrogen | Nitrate nitrogen | Ammoniacal nitrogen | Total mineral nitrogen | Organic nitrogen |
| SAMPLE 1. | | | | | | | | | | | | |
| 1 | 6.42 | 0.48 | 5.94 | 4.50 | 1.44 | 1.30 | 2.74 | 3.68 | 1.59 | 1.26 | 2.85 | 3.57 |
| 2 | 6.38 | 0.48 | 5.90 | 4.56 | 1.34 | 1.30 | 2.64 | 3.74 | 1.56 | 1.26 | 2.82 | 3.56 |
| 3 | 6.57 | 0.42 | 6.15 | 4.42 | 1.73 | 1.20 | 2.93 | 3.64 | 1.44 | 1.20 | 2.64 | 3.93 |
| 4 | 6.36 | 0.42 | 5.94 | 4.48 | 1.46 | 1.32 | 2.78 | 3.58 | 1.39 | 1.26 | 2.65 | 3.71 |
| 5 | 6.46 | 0.42 | 6.04 | 4.45 | 1.59 | 1.25 | 2.84 | 3.62 | 1.51 | 1.25 | 2.76 | 3.70 |
| 6 | 6.53 | 0.42 | 6.11 | 4.48 | 1.63 | 1.19 | 2.82 | 3.71 | 1.76 | 1.19 | 2.95 | 3.58 |
| 7 | 6.47 | 0.43 | 6.04 | 4.25 | 1.79 | 1.10 | 2.89 | 3.58 | 1.62 | 1.10 | 2.72 | 3.75 |
| 8 | 6.30 | 0.43 | 5.87 | 4.34 | 1.53 | 1.22 | 2.75 | 3.55 | 1.47 | 1.19 | 2.66 | 3.64 |
| 9 | 6.30 | 0.42 | 5.88 | .. | 1.46 | 1.14 | 2.60 | 3.70 | 1.80 | 1.12 | 2.92 | 3.38 |
| 10 | 6.33 | 0.45 | 5.88 | 4.54 | 1.34 | 1.21 | 2.55 | 3.78 | 1.47 | 1.18 | 2.65 | 3.68 |
| 11 | 6.32 | 0.49 | 5.83 | 4.50 | 1.33 | 1.18 | 2.51 | 3.81 | 1.45 | 1.18 | 2.63 | 3.69 |
| 12 | 6.28 | 0.52 | 5.76 | 4.42 | 1.34 | 1.36 | 2.70 | 3.58 | 1.68 | 1.29 | 3.02 | 3.26 |
| 13 | 6.25 | 0.37 | 5.88 | 4.22 | 1.66 | 1.18 | 2.84 | 3.41 | 1.67 | 1.21 | 2.88 | 3.37 |
| 14 | 6.17 | 0.46 | 5.71 | 4.15 | 1.56 | 1.21 | 2.77 | 3.44 | 1.64 | 1.21 | 2.85 | 3.36 |
| 15 | 6.21 | 0.41 | 5.80 | 4.17 | 1.63 | 0.95 | 2.58 | 3.64 | 1.70 | 0.95 | 2.65 | 3.56 |
| 16 | 6.43 | 0.43 | 6.00 | 4.33 | 1.67 | 1.12 | 2.79 | 3.64 | 1.75 | 1.13 | 2.88 | 3.55 |
| Averages | 6.36 | 0.44 | 5.92 | 4.39 | 1.53 | 1.20 | 2.73 | 3.63 | 1.59 | 1.19 | 2.78 | 3.58 |
| Calculated values | 6.35 | | | | 1.59 | 1.04 | 2.63 | 3.72 | 1.59 | 1.04 | 2.63 | 3.72 |
| SAMPLE 2. | | | | | | | | | | | | |
| 1 | 4.62 | 0.75 | 3.87 | 2.37 | 1.50 | 1.33 | 2.83 | 1.79 | 1.71 | 1.26 | 2.97 | 1.65 |
| 2 | 4.62 | 0.75 | 3.87 | 2.38 | 1.49 | 1.33 | 2.82 | 1.80 | 1.65 | 1.26 | 2.91 | 1.71 |
| 3 | 4.44 | 0.64 | 3.80 | 2.21 | 1.59 | 1.00 | 2.59 | 1.85 | 1.83 | 1.12 | 2.95 | 1.49 |
| 4 | 4.55 | 0.67 | 3.88 | 2.38 | 1.50 | 1.30 | 2.80 | 1.75 | 1.61 | 1.32 | 2.93 | 1.62 |
| 5 | 4.52 | 0.66 | 3.86 | 2.25 | 1.61 | 1.18 | 2.79 | 1.73 | 1.70 | 1.18 | 2.88 | 1.64 |
| 6 | 4.58 | 0.73 | 3.85 | 2.24 | 1.61 | 1.08 | 2.69 | 1.89 | 1.88 | 1.08 | 2.96 | 1.62 |
| 7 | 4.63 | 0.69 | 3.94 | 2.18 | 1.76 | 1.08 | 2.84 | 1.79 | 1.77 | 1.08 | 2.85 | 1.78 |
| 8 | 4.45 | 0.71 | 3.74 | 2.28 | 1.46 | 1.08 | 2.54 | 1.91 | 1.79 | 1.18 | 2.97 | 1.48 |
| 9 | 4.68 | 0.72 | 3.96 | .. | 1.71 | 0.95 | 2.66 | 2.02 | 1.73 | 1.06 | 2.79 | 1.89 |
| 10 | 4.51 | 0.69 | 3.82 | 2.43 | 1.39 | 1.20 | 2.59 | 1.92 | 1.54 | 1.15 | 2.69 | 1.82 |
| 11 | 4.54 | 0.74 | 3.80 | 2.48 | 1.32 | 1.18 | 2.50 | 2.04 | 1.66 | 1.18 | 2.84 | 1.70 |
| 12 | 4.60 | 0.78 | 3.82 | 2.34 | 1.48 | 1.22 | 2.70 | 1.90 | 1.73 | 1.23 | 3.01 | 1.59 |
| 13 | 4.43 | 0.65 | 3.78 | 2.15 | 1.63 | 1.13 | 2.76 | 1.67 | 1.66 | 1.16 | 2.82 | 1.61 |
| 14 | 4.57 | 0.69 | 3.88 | 2.24 | 1.64 | 1.12 | 2.76 | 1.81 | 1.73 | 1.12 | 2.85 | 1.72 |
| 15 | 4.38 | 0.69 | 3.69 | 2.12 | 1.57 | 0.89 | 2.46 | 1.92 | 1.74 | 0.89 | 2.63 | 1.75 |
| 16 | 4.51 | 0.78 | 3.73 | 2.11 | 1.62 | 1.05 | 2.67 | 1.84 | 1.80 | 1.10 | 2.90 | 1.61 |
| Averages | 4.54 | 0.71 | 3.83 | 2.28 | 1.56 | 1.13 | 2.69 | 1.85 | 1.72 | 1.15 | 2.87 | 1.67 |
| Calculated values | 4.42 | | | | 1.59 | 1.04 | 2.63 | 1.79 | 1.59 | 1.04 | 2.63 | 1.79 |

The collaborators were the following:

- (1) C. H. Jones, (2) G. F. Anderson, Agricultural Experiment Station, Burlington, Vt.
- (3) E. P. Bartlett and Mrs. Murray, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.
- (4) E. F. Boyce and L. S. Walker, Agricultural Experiment Station, Burlington, Vt.
- (5) J. H. Elder, (6) W. J. Franklin, (7) R. P. Hudson, Department of Agriculture, Richmond, Va.
- (8) M. P. Etheredge, Mississippi State Chemical Laboratory, A. & M. College, Miss.
- (9) A. H. Allen, Virginia-Carolina Chemical Corp., Richmond, Va.
- (10) W. D. Richardson, Swift and Co., Chicago, Ill.
- (11) A. O. Olson, Dairy and Food Department, St. Paul, Minn.
- (12) H. H. Hanson and R. E. Dickey, State Board of Agriculture, Dover, Del.
- (13) A. C. Wark, Agricultural Experiment Station, New Brunswick, N. J.
- (14) J. E. Breckenridge, American Agricultural Chemical Co., Carteret, N. J.
- (15) B. F. Robertson, Clemson Agricultural College, Clemson, S. C.
- (16) A. L. Prince.

After the collaborative study had been completed it was found desirable to change the wording of section 4 of the Robertson method in such a manner as to insist on the addition of mercury after the water is evaporated and white fumes appear. This seemed to be the safest procedure in order to include samples that contain much organic matter. However, in this connection one of the collaborators made the following important comment: "If the mercury is added too quickly, our nitrate nitrogen will show correspondingly low, but if the sample is boiled for a few minutes after the dense white fumes have appeared before the mercury is added, then the nitrate nitrogen figure seems to be more in line".

The data from the collaborative study have been compiled in Tables 1 and 2; the figures are averages of duplicate or triplicate determinations. At the bottom of each table are given the general average of all the collaborators for each particular form of nitrogen determined and the calculated values of the samples in question.

DISCUSSION.

In Table 1 are given the analytical results of sample No. 1 and sample No. 2 by the Robertson and Jones methods. The total nitrogen, given in column 1, was run by the regular Kjeldahl method to include nitrates and introduces nothing new. The collaborative results on the total nitrogen content are in quite close agreement, as would be expected, and the general average is practically identical with the calculated value. In columns 2 and 3 are reported the water-insoluble and water-soluble nitrogen. The determination of the water-soluble nitrogen, which is a necessary part in the Robertson scheme for the separation of nitrate nitrogen, shows good collaborative results. In column 4 is recorded the nitrogen after the ferrous sulfate treatment. This determination is made

TABLE 2.
Percentage of nitrate nitrogen.
 (Calculated value 1.59 %.)

| COLLABORATOR NO. | JONES METHOD | | ROBERTSON METHOD | | MODIFIED ROBERTSON METHOD | |
|---------------------|-----------------|-----------------|------------------|-----------------|------------------------------|-----------------|
| | Sample No. 1 | Sample No. 2 | Sample No. 1 | Sample No. 2 | Sample No. 1 | Sample No. 2 |
| 1 | 1.59 | 1.71 | 1.44 | 1.50 | 1.55 | 1.64 |
| 2 | 1.56 | 1.65 | 1.34 | 1.49 | | |
| 3 | 1.44 | 1.83 | 1.73 | 1.59 | 1.77 | 1.47 |
| 4 | 1.39 | 1.61 | 1.46 | 1.50 | 1.46 | 1.65 |
| 5 | 1.51 | 1.70 | 1.59 | 1.61 | 1.68 | 1.67 |
| 6 | 1.76 | 1.88 | 1.63 | 1.61 | | |
| 7 | 1.62 | 1.77 | 1.79 | 1.76 | 1.72 | 1.91 |
| 8 | 1.47 | 1.79 | 1.53 | 1.46 | 1.79 | 1.65 |
| 9 | 1.80 | 1.73 | 1.46 | 1.71 | 1.40 | 1.73 |
| 10 | 1.47 | 1.54 | 1.34 | 1.39 | 1.24 | 1.22 |
| 11 | 1.45 | 1.66 | 1.33 | 1.32 | | |
| 12 | 1.70 | 1.77 | 1.34 | 1.48 | 1.46 | 1.52 |
| 13 | 1.67 | 1.66 | 1.66 | 1.63 | | |
| 14 | 1.64 | 1.73 | 1.56 | 1.64 | 1.42 | 1.44 |
| 15 | 1.70 | 1.74 | 1.63 | 1.57 | | |
| 16 | 1.75 | 1.80 | 1.67 | 1.62 | 1.61 | 1.56 |
| Average | 1.59 | 1.72 | 1.53 | 1.56 | 1.55 | 1.59 |

on a portion of the water-soluble extract and by treatment with ferrous sulfate the nitrate nitrogen is eliminated, but the rest of the water-soluble nitrogen remains. It is this portion of the water-soluble nitrogen that is reported in column 4, and the figures are in fair agreement. The difference between column 3 and column 4 gives the nitrate nitrogen, as may be seen in column 5. These figures should be compared with the nitrate figures, which are given in column 1 under the Jones method. It will be observed that the variation is about the same by both methods. The general average by the Jones method is exactly the same as the calculated value. The general average by the Robertson method is 0.06 per cent under the calculated value. On this sample, which contained both cyanamide and urea, the nitrate figures would indicate that the methods are equally reliable.

However, with sample No. 2, which contained 100 pounds per ton of cyanamide without the urea, the nitrate nitrogen data by these two methods show greater variation, as may be noted under the columns marked "nitrate nitrogen". By the Robertson method, the values run from 1.32 per cent nitrate nitrogen to 1.76 per cent; by the Jones method the variation is from 1.54 to 1.88 per cent. The general average by the Robertson method is 1.56 per cent and by the Jones method it is 1.72 per cent, with the calculated value 1.59. Since most of the collaborative results by the Jones method are slightly high on this sample, it would appear that the Robertson method is more accurate.

The ammoniacal nitrogen, or that which is distilled from magnesium oxide, presents no particular problem whether it is determined on the water extract by Robertson's procedure or by the Jones procedure and the average results are about the same. However, it will be noted that the results in all cases are somewhat higher than the calculated values. It is the opinion of the associate referee that this may be due to a slight action of the magnesium oxide on the water-soluble organic matter in the samples.

The mineral nitrogen reported in column 7 under Robertson's method and column 3 under the Jones method represents the sum of the nitrate and ammoniacal nitrogen, and hence the results reflect the same tendencies as those already indicated for nitrate and ammoniacal nitrogen. For example, the average total mineral nitrogen on sample No. 1 by the Robertson method is 2.73 per cent and by the Jones method it is 2.78 per cent. On sample No. 2 these figures are 2.69 and 2.87, respectively. Since the calculated value is 2.63, there is indication that on the average the Jones method runs a trifle high for mineral nitrogen.

The organic nitrogen, given in the last column, was obtained by subtracting the mineral nitrogen from the total nitrogen. It is apparent that if the mineral nitrogen is higher than the calculated value, then the organic nitrogen obtained by this method will be lower than the calculated value. That tendency shows up in the general average and makes the organic nitrogen by the Jones method a little lower than the calculated value. The variation from the calculated value by the Robertson method for the organic nitrogen is somewhat less.

In fairness to the Jones method it should be stated that in all cases variation from the calculated values is not large, but taken as a whole the variation from the calculated values by the Robertson method is smaller. Furthermore, it seemed to be the consensus of opinion among the collaborators that the Robertson method is a simpler procedure; also the Robertson scheme of separating the various forms of nitrogen met with general favor.

In Table 2 a comparison is made between the modified Robertson method and the regular Robertson and Jones methods for nitrate nitrogen. Only eleven collaborators reported results on this modified method, but the data are very gratifying. The figures are more erratic than those obtained either by the Jones or regular Robertson method, but some workers obtained results very close to the calculated values. The general average on sample No. 1 is 1.55 per cent nitrate nitrogen and 1.59 per cent on sample No. 2. One cause for the greater variation among collaborators may lie in the fact that a sample of only 0.5 gram was used for analysis. Of course this necessitates more care in weighing and mixing. This modification shortens the procedure greatly, but it can be applied to advantage only when nitrate nitrogen alone is to be

determined in the mixed fertilizer. When water-soluble nitrogen is to be determined, as is the practice in most control laboratories, it would be necessary to follow the regular Robertson method.

The collaborative results on this modification indicate that the procedure would be worth while as a short cut whenever it could be applied. Instead of introducing this modification as a new method, the associate referee believes it would be best to append it to the regular Robertson method and state under what conditions it may be used.

Since the regular Robertson method appears to be a simpler procedure than the Jones method, and equally, if not more, accurate, the associate referee believes that it would be wise to replace the present tentative Jones method by the Robertson method. This is suggested because it is the present practice to include only one method for the same determination in *Methods of Analysis*, A. O. A. C.

Since preparing this report, the associate referee's attention has been called to some interesting work on the Robertson method by R. S. Gifford of the American Cyanamid Company. He carried out some extensive tests on solutions containing various amounts of cyanamide and nitrate, as well as on fertilizers containing these ingredients. Theoretically, in the Robertson method, the quantity of nitrogen remaining after the digestion with ferrous sulfate should be equal to that originally taken minus the nitrate nitrogen. However, when only 2 grams of ferrous sulfate was used, as directed in the method, this amount of nitrogen was always found to be less, and the loss increased as the amount of organic nitrogen and of nitrates was increased in the mixtures. It was found, however, that this loss could be eliminated by increasing the amount of ferrous sulfate used to 10 grams.

Another point brought out was that decomposition of cyanamide or urea occurred when the solution was distilled with magnesium oxide. The loss amounted to 3 or 4 per cent of the organic nitrogen with cyanamide and to about 8 per cent with urea. These facts have been noted by the associate referee in previous work on these methods.

Gifford also found that when large quantities of ammonium sulfate were present in fertilizer mixtures (200 lbs. per ton) the distillation of only 100 cc., as prescribed in the present methods, was not sufficient to carry over all the ammonia. This point was also brought to the associate referee's attention by A. W. Clark and H. M. Ellis of the New York Experiment Station at Geneva. These workers found that even a distillation of 200 cc. with caustic soda was not sufficient to remove all the ammonia when large amounts of ammonium sulfate were present. They believe that at least 350 cc. should be distilled over. It is believed by the associate referee that this matter should be referred to a special referee.

In the light of these new findings, it would seem wise to continue a study of the Robertson method and to seek modifications for its improvement. As it is the best available method for the determination of mineral and organic nitrogen in fertilizer mixtures containing nitrate, cyanamide or urea, it should be adopted tentatively at this time.

RECOMMENDATIONS¹.

It is recommended—

(1) That the Robertson method for the determination of nitrate nitrogen in mixed fertilizer containing cyanamide or urea be adopted as a tentative method to replace the present tentative Jones method.

(2) That the Jones modification of the Robertson method be appended to the Robertson method with the following note: "Applicable when water-soluble nitrogen is not needed".

(3) That the study of the Robertson method be continued and that various modifications be tried out with a view to securing further improvement.

REPORT ON NITROGEN ACTIVITY METHODS IN FERTILIZERS.

DETERMINATION OF ACTIVE WATER-INSOLUBLE NITROGEN BY THE ALKALINE PERMANGANATE METHOD².

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.),
Associate Referee.

In continuation of the study of the alkaline permanganate method for the activity of water-insoluble nitrogen in fertilizers³, three details received attention. Two are points of technic: the preparation of the alkaline permanganate solution and the method of transferring the washed residue from the filter to the flask. The third point concerns the adaptability of the process to the nitrogen of uric acid.

PREPARATION OF THE ALKALINE PERMANGANATE SOLUTION.

In a previous report⁴ it was shown that the quantity of ammonia distilled from protein materials digested in the alkaline permanganate solution depends to a large extent on the concentration of potassium permanganate in the solution, and that the solution is unstable. From subsequent collaborative work⁵, it was noted that solutions made in

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 70 (1930)

² Contribution 387 of the Rhode Island Agricultural Experiment Station.

³ *Methods of Analysis*, A. O. A. C., 1925, 12.

⁴ *This Journal*, 11, 191 (1928).

⁵ *Ibid.*, 12, 182 (1929).

accordance with the directions of the method varied from 21.2 to 24 grams per liter. The results for activity reported by the collaborators were correlated to a considerable degree with the oxidizing powers of the solutions.

Owing to the imperative need for a simple, but reasonably accurate, process for the preparation of the alkaline permanganate solution, a plan was devised and the following experiment was conducted: Two stock solutions were prepared; one with 300 cc. of sodium hydroxide per liter, and the other with 50 grams of C. P. potassium permanganate per liter. These solutions are of double strength for the method, and, when mixed in equal quantities, should make a solution conforming to the specifications for the procedure.

Early in January, 250 cc. of each solution was placed in a 500 cc. measuring flask, the contents were mixed, 6 cc. of water was added to replace the volume lost from the contraction due to solution of the two liquids, and the solution was transferred to a brown glass bottle. The oxidizing strength of the solution was determined by titration with 0.5 gram of C. P. sodium oxalate in 300 cc. of water and 10 cc. of concentrated sulfuric acid, and was found to be equivalent to 25.1 grams of potassium permanganate per liter. A second titration two weeks later showed a reduction in strength to 23.8 grams, and subsequent titrations at irregular intervals continued to show losses until, after standing for 9 months, the concentration was equivalent to only 22.2 grams per liter.

On the other hand, fresh mixtures of the two stored stock solutions, made at each of the above titration dates, and of the stock solution of potassium permanganate, showed full retention of strength and satisfactory uniformity during the nine months' period. The results are reported in Table 1.

With the future of the method in mind, refinement in titration methods was purposely sacrificed in the belief that sufficient accuracy can be obtained without the expense in time that more exact methods would require. The oxalate was dried and weighed carefully, but the titrations were conducted with a 10 cc. pipet of the Mohr type, rather than with a buret. There was, however, sufficient agreement between duplicate results to insure the success of the process in the hands of other analysts.

The contraction in volume when the two solutions are mixed is approximately 1 per cent of the desired volume. This error probably is not significant, but it can be corrected by the addition of 1 cc. of water for each 100 cc. of mixture.

This procedure is simple and rapid, and it will be uniform if the stock solution of permanganate is adjusted to contain 50 grams of potassium permanganate per liter by titration. One precaution must be observed.

The stock solution approaches saturation at 15°C., and for that reason should be stored above that temperature.

METHOD OF TRANSFER OF THE WASHED RESIDUE FROM FILTER TO KJELDAHL FLASK.

An important step in the method is the quantitative transfer of the leached residue of the sample from the filter paper to a Kjeldahl flask. As now written, the method requires that the residue be dried below 80°C. and then brushed into the flask, after which 20 cc. of water is added. In the previous report¹, a recommendation is noted from a collaborator, H. D. Haskins, advocating the use of the 20 cc. of water to wash the residue from the paper into the flask while the residue is wet, first transferring a large portion mechanically with a spatula or stirring rod. This procedure saves time, as no drying is required. The writer has not found this technic described in publications of the method, but has learned that it has been a common practice in some laboratories.

TABLE 1.

Stability of the alkaline permanganate solutions.*

| | SOLUTION A KMnO ₄ | SOLUTION B KMnO ₄ | SOLUTION C KMnO ₄ |
|------------|---------------------------------|---------------------------------|---------------------------------|
| | grams per liter | grams per liter | grams per liter |
| 1929 | | | |
| January 12 | 50 2 | 25 1 | |
| January 28 | 50 3 | 23 8 | 25 4 |
| February 9 | 49 6 | 23 3 | 24 8 |
| March 26 | 49 6 | 22.9 | 24 6 |
| June 20 | 51 3 | 22 9 | 25 6 |
| October 4 | 51 2 | 22 2 | 25 4 |

* Solution A was made January 12.

Solution B was made January 12, by diluting A with an equal quantity of a solution containing 300 grams of NaOH per L.

Solution C similar to B, but these are fresh solutions made on each successive titration date.

TABLE 2.

Comparison of the quantities of nitrogen transferred from filter to flask by (A) washing in the wet residue with 20 cc. of water, aided by brushing with a spatula; and (B) brushing in the dried residue with a stiff brush.

| SAMPLE NO. | A | B | SAMPLE NO. | A | B |
|------------|------|------|------------|------|------|
| | mg. | mg. | | mg. | mg. |
| 1 | 49 9 | 50 0 | 11 | 49 9 | 50 0 |
| 2 | 50.0 | 49 8 | 12 | 48 5 | 51 0 |
| 3 | 50.0 | 50 1 | 13 | 50 0 | 49 8 |
| 4 | 49.8 | 49 6 | 14 | 49 9 | 49.8 |
| 5 | 49 7 | 49 3 | 15 | 50 1 | 50.0 |
| 6 | 49.9 | 49 7 | 16 | 49 9 | 49 8 |
| 7 | 50 0 | 49 2 | 17 | 49 9 | 49 6 |
| 8 | 50.0 | 50 1 | 18 | 49.9 | 49 6 |
| 9 | 50.7 | 50 6 | 19 | 49 9 | 50 0 |
| 10 | 50.6 | 49.9 | 20 | 50 0 | 49 5 |

Average—Method A—49 93 mg. N.

Average—Method B—49 87 mg. N.

¹ *This Journal*, 12, 182 (1929).

To compare the accuracy of the two procedures, samples of 20 mixed fertilizers were weighed in duplicate on two filter papers. The quantities taken were calculated to contain 50 mg. of water-insoluble nitrogen. Each sample was leached with 250 cc. of water. One was then transferred immediately to a Kjeldahl flask by spreading the paper on a metal disk, bent to fit the palm of the hand, brushing a large portion of the wet residue into the flask with a spatula, and then washing in the remainder with 20 cc. of water. This was done conveniently by constructing a small wash bottle from a 20 cc. graduate. The second residue was dried below 80°C. and brushed from the paper into a flask by means of a stiff bristled tube brush. Total nitrogen was then determined in the material in the flasks and the quantity transferred was calculated in milligrams.

The results, reported in Table 2, are in close accord and show no material difference between the two methods. The odds, calculated by the well-known "Student's" method, that transfer of the wet residue will place the greater quantity of nitrogen in the flask, are only 2 : 1. These are considered very low odds and indicate no significant difference between the methods. Since each has advantages under different conditions, both practices should be allowed.

No data were secured concerning the effect of drying below 80°C. on the activity of the nitrogen as compared with that of the wet material, but there seems no reason to expect error from that source.

APPLICATION OF THE METHOD TO URIC ACID.

Moore and White¹ directed attention to the fact that the alkaline permanganate method showed an activity of only 37 per cent for the insoluble nitrogen of Peruvian guano, while by the neutral permanganate method the activity was 97 per cent. More recently, Haskins² compared the activities of the insoluble nitrogen in uric acid by the alkaline and by the neutral permanganate methods, with the availability as shown by pot tests. Both from the weight of crop grown, and from the quantity of nitrogen removed from the soil by the crop, the uric-acid nitrogen received an availability rating of 90, as compared to the conventional standard of 80 for dried blood. The activity by the neutral method was 83.2, but that by the alkaline method was only 23.6.

The evidence seems conclusive that the alkaline method is not applicable to the nitrogen in uric acid, and that for mixtures containing this ingredient, only the neutral method should be used.

¹ *This Journal*, 10, 202 (1927).

² *Am. Fertilizer*, 70, No. 5: p. 23.

RECOMMENDATIONS¹.

The following changes are recommended in *Methods of Analysis*, A. O. A. C., 1925, and in the revision published in *This Journal*, 11, 33 and adopted finally in 1928, *This Journal*, 12, 33:

1. Paragraph 40. Delete the revised instructions for the preparation of the alkaline permanganate solution as published in *This Journal*, 12, 33 and substitute the following:

(a) *Stock solution of potassium permanganate*.—Dissolve 50 grams of potassium permanganate in a liter of water. Dissolve 0.5 gram of sodium oxalate in 300 cc. of water and 10 cc. of concentrated sulfuric acid. Heat to 75°–80°C. and titrate with the potassium permanganate solution, using a Mohr pipet or an all-glass buret to contain the permanganate solution. 235.89 divided by the result of the titration in cubic centimeters gives the concentration of potassium permanganate in grams per liter. Adjust the concentration to 50 grams per liter. Store at a temperature above 15°C.

(b) *Stock solution of sodium hydroxide solution*.—Dissolve 300 grams of sodium hydroxide in a liter of water. Cool before use.

(c) *Alkaline permanganate solution*.—Mix equal quantities of the stock solutions of potassium permanganate and sodium hydroxide, and add 10 cc. of water for each liter of solution that the mixture is calculated to make. Make fresh each day, as the solution is unstable.

2. Paragraph 42. (Revised in *This Journal*, 11, 34). Following the second paragraph of this section, add:

Previous to digestion with alkaline permanganate the washed sample may be transferred from the filter to the flask without drying by spreading the wet filter on a metal disk bent to form a trough that fits the palm of the hand, brushing the larger portion of the material into the flask with a spatula, and washing in the remainder with 20 cc. of water from a 20 cc. pipet or small wash bottle. Do not add more water before the digestion with alkaline permanganate, but, with that exception, proceed as with the transfer of the dried material.

REPORT ON POTASH.

By L. D. HAIGH (Agricultural Experiment Station, Columbia, Mo.),
Associate Referee.

It was recommended by Subcommittee A that the associate referee take up the study of methods for potash in mixed fertilizers. In accordance with this recommendation it was suggested that the Fraps method² be studied in comparison with the regular official method. The associate referee has made some preliminary trials with this method during the year, but does not feel that the work has progressed far enough to make any definite report. He would therefore recommend that further time be given to work out details of the Fraps method before a collaborative study is undertaken.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 80 (1930).

² *This Journal*, 9, 193 (1926).

REPORT ON PLANTS.

By O. B. WINTER (Agricultural Experiment Station, East Lansing, Mich.), *Referee*.

As was recommended by the association last year, the work on plants for the current year was conducted along the following lines: (1) Preparation of material for analysis; (2) determination of the less common metals; (3) determination of total chlorine; (4) consideration of methods for the determination of carbohydrates and of the various forms of nitrogen; and (5) microchemical methods for the determination of iron and aluminum.

Associate referees were appointed to supervise the work on the first three projects, and their reports deserve the careful attention of the association. No work was done on the fourth project, but several laboratories have shown such a keen interest in it that associate referees should be appointed for that work. The referee directed the work on the fifth project. The report of this work follows.

IRON AND ALUMINUM IN PLANTS.

For the collaborative work, four samples accompanied in part by the following instructions were sent to each collaborator.

Under separate cover I am sending you four A. O. A. C. samples to be analyzed for iron and aluminum by a method proposed for the determination of iron and aluminum in plants: 1. Aluminum solution, 2. Iron and aluminum solution, 3. Plant material, and 4. Plant material.

In order that you may be able to make the desired determinations with as little waste of time as possible, I am making the following suggestions concerning each sample:

Sample No. 1, a synthetic solution, is to be run for aluminum only. Take 5 cc. of the sample and proceed as under the "Determination of Aluminum".

Sample No. 2 is a synthetic solution, the iron and aluminum of which are to be separated and each determined. Take 15 cc. of the sample. After separating the iron and aluminum take 10 cc. aliquots for determining the aluminum, and after dissolving the iron hydroxide take one-half of the solution for determining the iron.

Sample No. 3. Burn a 1 gram sample. After making the iron and aluminum separation, take 2 cc. aliquots for the aluminum determination, and after dissolving the iron hydroxide take one-half of the solution for the iron determination.

Sample No. 4. Burn a 5 gram sample. Proceed as under No. 2 after making the iron and aluminum separation.

METHODS FOR THE DETERMINATION OF IRON AND ALUMINUM.

REAGENTS.

- (a) *Hydrochloric acid*.—1.5 N and 6 N.
- (b) *Ammonium acetate solution*.—5 N.
- (c) *Ammonium carbonate solution*.—3 N.
- (d) *Ammonium chloride solution*.—5 N.
- (e) *Ammonium hydrogen phosphate solution*.—10 per cent.
- (f) *Ammonium salt of aurin tricarboxylic acid solution*.—0.1 per cent.

- (g) *Ammonium sulfocyanate solution*.—15 per cent.
- (h) *Standard aluminum solution*.—1 cc. contains 0.01 mg. of aluminum.
- (i) *Standard iron solution*.—1 cc. contains 0.05 mg. of iron.

PREPARATION OF SAMPLE.

Place from 1–30 grams of material (depending on the quantity of aluminum present) in a platinum dish in an electric muffle, raise the temperature to just below redness, and allow to remain overnight. (Ignore any unburned carbon at this point, since it may be ignited later.) Digest with dilute hydrochloric acid, transfer to a centrifuge tube, centrifuge, and decant the supernatant liquid into a Pyrex beaker. Call this solution A. Transfer the residue to a platinum crucible by means of a fine jet of water, evaporate to dryness, ignite if necessary, and fuse with a mixture of 1 gram of equal parts of sodium and potassium carbonates. Take up with hydrochloric acid and add to solution A.

SEPARATION OF IRON AND ALUMINUM.

After oxidizing the iron in Solution A with nitric acid and removing the silica by dehydration, take up with hydrochloric acid and transfer the solution to a centrifuge tube of about 30 cc. capacity with marks at 15 cc. and 25 cc. Dilute to about 15 cc. with distilled water, add 1 cc. of 10 per cent ammonium hydrogen phosphate and neutralize with dilute ammonium hydroxide, using thymol blue (acid range) as the indicator. Add 1 cc. of a saturated solution of ammonium acetate and place the tube in a steam bath until the precipitate flocculates (about 10 minutes). Cool, centrifuge, decant, and discard the supernatant liquid. Wash once with 5 cc. of 5 per cent ammonium nitrate solution by means of the centrifuge and decantation. (This is best done by adding about 2 cc., shaking to break up the precipitate, and then washing down the sides of the tube with the remaining 3 cc.)

Dissolve the precipitate in 1 cc. of 6 *N* hydrochloric acid and dilute to about 15 cc. To this solution, cooled to room temperature, add 1.25 cc. of glacial acetic acid, 5 cc. of 6 *N* sodium hydroxide solution prepared from metallic sodium (special aluminum free), and add water to the 25 cc. mark. Allow to stand with an occasional shaking for about 1 hour and then centrifuge. The precipitate contains the iron and the supernatant liquid the aluminum.

DETERMINATION OF ALUMINUM.

Transfer an aliquot of the solution to a 50 cc. graduated flask and add a small piece of litmus paper, water to make a volume of about 20 cc. and dilute hydrochloric acid until the litmus paper just turns red. Determine the aluminum according to the colorimetric method by Winter, Thrun and Bird¹.

DETERMINATION OF IRON.

Determine the iron by the colorimetric method given by Patten and Winter in *J. Assoc. Official Agr. Chem.*, 11, 207 (1928).

REMARKS.

Correct the results for aluminum by the aluminum content of a blank on all the reagents used.

Do not filter through paper—use the centrifuge. If this is not practicable, the blanks must be filtered through similar papers as the samples.

Do not use rubber policeman to remove precipitates from crucibles, etc.

¹ *J. Am. Chem. Soc.*, 51, 2730 (1929). The details of the method, including the graph, were given in this letter. However, since these have been published elsewhere, only the reference is given here.

The results obtained by the different collaborators are found in Table 1.

TABLE 1.
Iron and aluminum in plants.

| COLLABORATOR | IRON | | | ALUMINUM | | | |
|------------------|----------------|-----------------|-----------------|----------------|----------------|-----------------|-----------------|
| | No. 2 | No. 3 | No. 4 | No. 1 | No. 2 | No. 3 | No. 4 |
| | <i>mg./cc.</i> | <i>per cent</i> | <i>per cent</i> | <i>mg./cc.</i> | <i>mg./cc.</i> | <i>per cent</i> | <i>per cent</i> |
| 1 | 0.054 | 0.0044 | 0.0042 | 0.0100 | 0.0080 | 0.039 | 0.0064 |
| | 0.050 | 0.0047 | 0.0041 | 0.0096 | 0.0083 | 0.037 | 0.0072 |
| | 0.051 | 0.0044 | 0.0045 | 0.0092 | 0.0078 | 0.038 | 0.0066 |
| | 0.052 | 0.0045 | 0.0043 | 0.0096 | 0.0080* | 0.038 | 0.0067* |
| 2 | | | | 0.0124* | 0.0067 | 0.041 | 0.0017 |
| 3 | 0.0507 | | | 0.0103 | 0.0060 | 0.0337 | |
| | 0.0500 | | | 0.0105 | 0.0062 | 0.0320 | |
| | 0.0504 | 0.0052 | | 0.0104 | 0.0061 | 0.0329 | 0.0015 |
| 4 | 0.0500 | | | | | | |
| | 0.0516 | | | | | | |
| | 0.0513 | | | | | | |
| | 0.0510 | 0.0056 | | 0.0098 | 0.0060 | 0.0318 | 0.0018 |
| 5 | | | | | | 0.0325 | 0.0015 |
| General average | 0.0518 | 0.0051 | | 0.0099 | 0.0063 | 0.0352 | 0.0016 |
| Quantity present | 0.0516 | | | 0.0100 | 0.0064 | | |

* Not included in the general averages.

A careful study of Table 1 shows that in sample No. 1, the synthetic solution which required no separation of iron and aluminum, the results for aluminum, with one exception, are entirely satisfactory. In sample No. 2, the synthetic solution which did require the separation of iron and aluminum, and in the samples of plant materials, the results for aluminum do not agree so well as might be desired. The results for the iron in sample No. 2 are very satisfactory, while those on the samples of plant ash are not quite so good. On the other hand, these data are very encouraging—possibly all one should expect when dealing with what used to be considered *bare traces*. They strengthen the referee's belief that colorimetric methods can be made sufficiently accurate for determining small amounts of iron and aluminum as well as of other elements present in plants.

The determination of iron by the method herein described involves certain difficulties. (1) It has been held that the presence of much phos-

phate prevents complete development of the ferric thiocyanate color and that a small amount of phosphate does not interfere with this color development. Hence, in the method used for iron determinations in plants given in this report, the iron and aluminum are precipitated as phosphates, while the other salts remain in solution. However, in samples very high in calcium phosphate and very low in iron and aluminum, the calcium phosphate is precipitated with the iron and aluminum phosphates. Fortunately the referee has not found this difficulty in plant materials. Nevertheless, it is desirable to have a method applicable to all materials. (2) Conditions have not been obtained under which the ferric thiocyanate color always remains sufficiently permanent to compare a number of samples with one standard. Some analysts have not mentioned this difficulty, while others find considerable trouble with it. (3) The use of sodium or potassium hydroxide for the separation of iron and aluminum is not entirely satisfactory. One difficulty is that the purest reagents obtainable contain traces of aluminum, and another is that conditions must be exactly right or the separation is incomplete.

The difficulties should be overcome if possible and more collaborative work should be done on the methods.

It might be stated here that work is being done in this laboratory on these problems, and the following observations may be reported: (1) The fading of the ferric thiocyanate color appears to be due to improper adjustment of the acidity of the solution, a reduction of the iron in the solution, or both; (2) there is a possibility of determining the iron colorimetrically without removing the calcium phosphate—even in such material as milk; (3) the method of removing the iron by extraction with ether, as was done by Underhill and Peterman¹ in determining aluminum in biological materials, is promising. This method would eliminate the use of sodium or potassium hydroxide for making the iron and aluminum separation.

RECOMMENDATIONS².

It is recommended—

- (1) That reports of the associate referees be adopted.
- (2) That an associate referee be appointed to study methods for the determination of carbohydrates in plants.
- (3) That an associate referee be appointed to study methods for the determination of the various forms of nitrogen in plants.
- (4) That the study of the methods for the determination of iron and aluminum be continued and that more collaborative work be done on them.

¹ *Am. J. Phys.*, **90**, 1 (1929).

² For report of Subcommittee A and action of the association, see *This Journal*, **13**, 61 (1930)

REPORT ON PREPARATION OF PLANT MATERIAL FOR ANALYSIS.

By H. R. KRAYBILL (Agricultural Experiment Station, Purdue, Ind.),
Associate Referee.

The method of preparation of the sample of plant material for analysis obviously will depend upon the constituents which are to be determined. If mineral constituents only are to be determined, the sample may be prepared satisfactorily by a method which would not be satisfactory if various carbohydrates or forms of nitrogen are to be determined. For this reason it seems advisable to have different methods of preparation of the sample for the following three types of determinations: (1) mineral constituents, (2) carbohydrates, (3) forms of nitrogen. It is recommended that the first paragraph in section 4 of Plants—Preparation of sample—Official, be replaced by the following:

1 DIRECTIONS FOR SAMPLING.¹

When more than one plant is sampled, a sufficient number of plants should be included in the sample to insure that it represents adequately the average composition of the entire lot of plants sampled. The number of plants necessary for an accurate sample cannot be stated definitely but will depend upon the variability in composition of the plants. Details of the procedure must be determined by the purpose for which the sample is taken.

2 PREPARATION OF SAMPLE.

(a) *For mineral constituents.*—Thoroughly remove all foreign matter from the material, especially adhering soil or sand, avoiding excessive washing to prevent leaching; air dry as rapidly as possible to prevent decomposition or loss in weight by respiration; grind; and preserve in tightly stoppered bottles. If it is desired to express the results on the fresh weight basis, the weights of the sample before and after air drying should be recorded. When determinations of copper, manganese, zinc, iron and aluminum are made, precautions should be taken to prevent contamination of the sample from dust during air drying and from the grinding and sieving machinery.

It is suggested that the following method of preparation of sample for carbohydrates be adopted tentatively and that studies be continued on this method.

(b) *For carbohydrates.*—Thoroughly remove all foreign matter from the material; grind or chop the material into fine pieces rapidly and add the weighed sample to sufficient hot redistilled 95 per cent alcohol to which sufficient precipitated calcium carbonate has been added to neutralize the acidity. Use sufficient alcohol so that the final concentration, allowing for the water content of the sample, will be approximately 80 per cent. Heat close to the boiling point on a steam or water bath with frequent stirring for 30 minutes. The samples may then be stored until ready for analysis.

During last year the associate referee studied the effect of preserving samples of plant material in 80 per cent alcohol without neutralization

¹ *Botanical Gazette*, 73, 44 (1922), *Proc. Am. Soc. Hort. Sci.*, 1927, p. 191.

of acidity upon the nitrate nitrogen, ammonia nitrogen, alpha amino acid nitrogen and amide nitrogen. These results have not yet been completed. Until more data are available it does not seem wise to adopt the alcohol method of preservation of samples. It is recommended that these studies be continued¹.

REPORT ON LESS COMMON METALS IN PLANTS.

By J. S. MCHARGUE (Department of Chemistry, Agricultural Experiment Station, Lexington, Ky.), *Associate Referee*.

Tentative methods for the determination of copper, manganese and zinc in plant material were adopted at the 1928 meeting, and it was thought advisable to do further cooperative work and to report the results obtained at the meeting this year. After correspondence with the Referee on Plants, a synthetic solution containing known quantities of copper, manganese, zinc, iron, aluminum, calcium, magnesium, phosphorus, potassium and sodium was made, and portions were sent to those chemists who had agreed to take part in the work. The concentration of the synthetic solution was such that 25 cc. contained approximately the amount and kind of mineral matter that is found in the ash from 100 grams of plant material. The results of the collaborative work accomplished this year are given in Table 1.

TABLE 1.

Collaborative results on the determination of copper, manganese and zinc in plant material.

| Synthetic solution | Copper | Manganese | Zinc |
|-------------------------------|-----------|-----------|----------|
| 1 cc. contained grams of. . . | 0 00008 | 0 0004 | 0 0004 |
| Collaborator | | | |
| 1 | 0 000081 | 0 00044 | 0 00024* |
| 2 | 0 000073 | 0 00040 | 0 00011* |
| 3 | 0 000068 | 0 00039 | 0 00033 |
| 4 | 0.000076 | 0.00039 | 0 00040 |
| 5 | 0 000080 | 0 00044 | 0 00038 |
| 6 | 0 000078 | 0 00049 | 0 00042 |
| 7 | 0 000110 | 0 00040 | 0.00032 |
| 8 | 0 000050* | 0 00040 | 0 00040 |
| Average | 0 000081 | 0 00042 | 0 00038 |

* Not included in the average.

The averages of the results reported by the various collaborators on the analyses of the synthetic solution indicate that the tentative methods for the determination of copper, manganese and zinc gave fairly concordant results. One of the results for copper was a little high and another was correspondingly low, therefore the average for copper is

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 61 (1930).

near the amount added. The results for manganese are excellent, the average in the fifth decimal place being two points above the amount added. Two of the collaborators experienced some difficulty on the determination of zinc and reported results so low that they were not included in the average. Two others reported low results, and the other four reported good results.

One of the difficulties encountered in the determination of zinc was the development of a bluish green color characteristic of traces of iron which tended to interfere with the turbidity readings. This difficulty was practically eliminated by using a more dilute solution (0.2 per cent) of potassium ferrocyanide and making the comparisons about 5-10 minutes after the addition of the ferrocyanide reagent. This change in the tentative methods is recommended. It is recommended that the methods for the determination of copper, manganese and zinc in plants be made official¹.

Some preliminary work has been done on methods for the determination of iodine in agricultural limestones, soils and forage crops. However, further study is desired before attempting a report on this subject, and it is recommended that attention be given to methods for the determination of iodine in soils, agricultural limestone, forage crops and foods during the next year.

REPORT ON TOTAL CHLORINE IN PLANTS.

By MORTON F. MASON (Agricultural Experiment Station, East Lansing, Mich.), *Associate Referee*.

Since the 1928 collaborative work did not show adequate agreement between methods, nor among the investigators using the same method, it appeared advisable to carry out the recommendations concerning the further study of methods and not the one asking for further collaborative work. Hence no collaborative work was planned.

Because the 1928 report included collaborative work done on pineapple juice only and because the major part of the discussion involved the analysis of known solutions of sugar and sodium chloride (i. e., sirups), the first step taken was to attempt a quantitative recovery of chlorine in such solutions. Later, work was done with materials such as corn, oats, tobacco, alfalfa, dried bovine feces, sirups from canned fruits, etc. A review of the literature on the determination of chlorine, most of which appeared in the *Journal of Biological Chemistry* (1915-1929), will not be given here.

Critical investigation has shown that silver nitrate may be titrated with thiocyanate in the presence of silver chloride and nitric acid, ferric

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 62 (1930).

alum being used as an indicator¹. Hence the procedure used by Van Slyke² was closely adhered to in the following preliminary analyses:

A sample containing not more than 20 mg. of chlorine in 3-10 cc. of sirup was added to 10-15 cc. of 0.05 *N* silver nitrate in concentrated nitric acid in a 100 cc. extraction flask. This was covered with a watch-glass, digested on a steam bath for 3 or 4 hours, or overnight, diluted with an equal volume of distilled water, cooled, and the excess of silver nitrate was titrated to a salmon pink with 0.05 *N* potassium thiocyanate after an addition of about 0.5 gram of ferric alum.

This method proved entirely satisfactory for samples which contained little carbohydrate, but, as stated in the 1928 report, as the concentration of sugar increased, the interference of the oxides of nitrogen with the end point introduced large errors. Upon the addition of the ferric alum an intense yellow-green color was produced, which made it difficult, if not impossible, to ascertain the end point when titrating with the thiocyanate. The analyses of six different sugar-salt solutions in Table 1 are typical of the results obtained.

TABLE 1.

Chlorine in sugar-salt solutions by the Van Slyke method.

| SAMPLE | SALT ADDED PER 100 CC. | SUGAR IN 100 CC. | CHLORINE FOUND | CHLORINE PRESENT |
|--------|---------------------------|---------------------|-------------------|---------------------|
| | <i>gram</i> | <i>grams</i> | <i>mg.</i> | <i>mg.</i> |
| 1 | 0.0998 | 0 | 61.1 | 60.5 |
| 2 | 0.0924 | 4 | 55.2 | 55.8 |
| 3 | 0.0686 | 10 | 40.7 | 41.6 |
| 4 | 0.2177 | 16 | 129.2 | 132.1 |
| 5 | 0.1627 | 24 | 95.2 | 98.7 |
| 6 | 0.2787 | 40 | 161.4 | 169.0 |

Attempts were made to sharpen the end point by diluting the digestion mixture with an equal volume of acetone before cooling and titrating as before, but without success. A saturated solution of hydrazine sulfate added to the digestion mixture just previous to titration, as recommended in the 1928 report, did not help appreciably. It was concluded that the simple Van Slyke digestion was not applicable to sirups.

The addition of solid potassium permanganate and digestion over a Bunsen burner³ were then tried, and they proved very effective. In solutions containing sufficient sugar to prevent the distinguishing of any end point whatsoever by the straight Van Slyke procedure, the addition of potassium permanganate made possible an accurate determination, although rather large quantities of the permanganate were sometimes necessary. Table 2 shows the results of the analyses of the last three samples given in Table 1 when potassium permanganate was added to assist digestion.

¹ Lawrence and Harris, *Am. Chem. J.*, 46, 1471 (1924).

² *J. Biol. Chem.*, 58, 523 (1923).

³ *Ibid.*, 82, 411 (1929).

TABLE 2.

Chlorine in Solutions 4, 5 and 6 of Table 1, by the Van Slyke method, with the addition of potassium permanganate.

| SAMPLE | CHLORINE FOUND |
|--------|----------------|
| | <i>mg.</i> |
| 4 | 132.0 |
| 5 | 99.3 |
| 6 | 168.0 |

The data in Tables 1 and 2 indicate that chlorine may be determined by the Van Slyke method with the addition of solid potassium permanganate during the digestion.

PROCEDURE.

Add a sample containing not more than 20 mg. of chlorine in 3–10 cc. of sirup to 10–15 cc. of 0.05 *N* silver nitrate in concentrated nitric acid in a 100 cc. extraction flask, add carefully about 1 gram of solid potassium permanganate (the reaction may be violent here), cover with a watch-glass, and boil over a Bunsen burner until the mixture almost clears (usually about 5 minutes). Cool, add another gram of potassium permanganate, and heat until the solution is clear. If a brown color persists, add sufficient ammonium oxalate to remove this color, heat on the steam bath until the silver chloride flocks out in relatively large masses, dilute with an equal volume of water, cool in running water, and titrate any excess silver nitrate with potassium thiocyanate in the presence of ferric alum.

It is believed that virtually all plant materials will be sufficiently oxidized through this treatment to render the titration possible. A singular exception was peat, which remained a deep brown colloidal mass in spite of heavy additions of potassium permanganate and long-continued boiling.

Determinations made in this manner were checked by the dry ashing method, followed by the Volhard titration, as proposed in the 1928 report. Table 3 shows a number of comparative results.

TABLE 3.

Comparative chlorine determinations.

| SAMPLE | CHLORINE ADDED AS SALT | CHLORINE FOUND | | CHLORINE PRESENT |
|---------------------------------------|------------------------------|--------------------------------|---|---------------------|
| | | Van Slyke + KMnO_4 | Dry Ashing with Na_2CO_3 | |
| | <i>mg.</i> | <i>mg.</i> | <i>mg.</i> | <i>mg.</i> |
| 5% sugar solution | 60.5 | 61.1 | 61.2 | 60.5 |
| 40% " " " " " " " " " " | 169.0 | 168.0 | 168.0 | 169.0 |
| Unsweetened pineapple juice | " " | 23.1 | 23.5 | " " |
| Pineapple sirup | " " | 19.5 | 19.3 | " " |
| " " " " " " " " " " | 60.5 | 80.6 | 80.6 | 80.0 |
| Apricot sirup | " " | Trace | Trace | " " |
| " " " " " " " " " " | 60.5 | 61.4 | 60.3 | 60.5 |

The results given in Table 3 show that the Van Slyke method with potassium permanganate and the dry ashing method with sodium carbonate agree very closely.

The adaptability of the procedure to analyses of a few aromatic halogen derivatives was investigated. The determination could be made in most cases, although certain compounds so highly colored the digestion mixture (and could not be broken down further with potassium permanganate) that the back titration was impossible. As these compounds were class-room preparations of questionable purity, the accuracy of the determinations could not be checked except that additional chlorine added as sodium chloride was quantitatively recovered. Further investigation on pure compounds is necessary.

SUMMARY.

1. The open Carius digestion recommended by Van Slyke et al. for chlorine in biological materials is impractical when the sample contains large amounts of carbohydrates.

2. The addition of solid potassium permanganate before and during digestion makes the analysis of sirups, corn, oats, wheat, tobacco, alfalfa, dried feces, etc., possible.

3. The open Carius digestion method, using potassium permanganate, checks the dry ashing method within experimental error.

4. Preliminary work on chlorine in impure organic compounds indicated that accurate results are possible.

RECOMMENDATIONS¹.

It is recommended—

(1) That collaborative work be done on a number of samples of plant material including sirups by using the open Carius digestion with the addition of solid potassium permanganate.

(2) That additional work be done on the determination of chlorine in pure organic compounds by this method.

REPORT ON DAIRY PRODUCTS.

By HERMANN C. LYTHGOE (Department of Public Health, Boston, Mass.), *Referee*.

No report will be presented by B. G. Hartmann, Associate Referee on Malted Milk, who recommends that the study of methods for the determination of lactose be dropped and that the collaborative study of the microscopical identification of malted milk be continued. E. L. P. Treuthardt, Associate Referee on Dry Milk, presents a comprehensive report with a few minor suggestions as to changes in the methods, which suggestions are approved by the referee. Henry Hoffmann, Jr., presents reports on Sediment in Milk and on Butter. Erwin C. Huebner pre-

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 62 (1930).

sents a report on cheese. The other associate referees made no reports.

A short time ago the referee discussed with Phillip H. Smith of the Massachusetts Agricultural Experiment Station, who is in charge of the work relative to the calibration of glassware for use in the Babcock machine in Massachusetts, the multiplicity of the Babcock bottles for cream approved by this association. It was deemed advisable to ascertain whether or not there was any demand for the adoption or the retention of more than one type of test bottle for cream. This action is particularly desirable at the present time since in the State of Massachusetts, for example, only one type of Babcock cream-test bottle is legal. The following letter was therefore sent to the various experiment stations, food and drug commissions, boards of health and to a few milk inspectors of Massachusetts:

Gentlemen:

There has been suggested to me the advisability of the Association of Official Agricultural Chemists in its official methods recommending only one type of bottle for the determination of fat in cream by the Babcock method. Your criticism of this suggestion is requested, and I furthermore request, if you feel it advisable for the Association to adopt the above suggestion, that you kindly state which bottle should be made the standard bottle of the Association. I have been informed that the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle has been adopted by the Bureau of Standards as their standard bottle.

The concensus of opinion seems to be in favor of the adoption of but one type of bottle, that one being the 9 gram, 50 per cent, 6 inch, short neck bottle.

Replies were received in part as follows:

A. D. Burke, Head of Dairy Department, Agricultural Experiment Station, Auburn, Alabama.—I see no particular reason for having two or three types listed.

H. E. Dvorachek, Head of Department of Animal Industry, Agricultural Experiment Station, Fayetteville, Arkansas.—I am heartily in accord with such a recommendation. I believe the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle would be the most desirable, in view of the fact that it is the most commonly used, and also has been adopted by the Bureau of Standards as their standard bottle.

Frank T. Shull, Dominion Chemist, Central Experiment Farm, Ottawa, Ontario.—I certainly think it would be desirable to have only one type of bottle for the determination of fat in cream by the Babcock method—and I should be strongly in favor of the A. O. A. C. adopting the standard bottle of the Bureau of Standards.

Thomas Holt, Commissioner, Dairy and Food Commission, Hartford, Connecticut.—The one that you describe is the only one that is allowed by this department.

T. A. Baker, Professor of Dairy Husbandry, University of Delaware.—We are using the cream-test bottle which is adopted by the Bureau of Standards, and we believe this to be the most satisfactory.

F. R. Edwards, Animal Husbandman, Georgia Experiment Station.—We consider that the advantages from using only one type of bottle instead of several types, as is now the case, would outweigh the disadvantages and accordingly consider that such a step would be advisable. We recommend, if only one type of bottle be taken as a standard, that the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle be used.

D. R. Theophilus, Associate Professor, Dairy Manufacturers, University of Idaho.—I, personally, feel that it is advisable for the Association of Official Agricultural Chemists in its official methods to recommend only one type of bottle for the determination of fat in cream by the Babcock method. * * * A considerable number of the states have only one standard cream-test bottle and I see no reason why it should not be uniform, as my experience does not indicate that other cream-test bottles are more accurate than the 9 gram, 50 per cent, 6 inch cream-test bottle, graduated to 0.5 per cent.

H. A. Ruehe, Head of the Department of Dairy Husbandry, Agricultural Experiment Station, Urbana, Illinois.—There are times, however, when some farmers do deliver cream testing over 50 per cent. The percentage of farmers that do this is relatively small, however. I am of the opinion that the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle in most respects is the most satisfactory cream-test bottle to use.

J. L. Miller, State Food and Drug Commissioner, Indiana.—The 9 gram, 50 per cent, short neck, 6 inch cream-test bottle has been standard for this State for several years.

Carrell H. Whitnah, Dairy Chemist, Kansas State Agricultural College.—We use the 9 gram, 50 per cent, short neck cream-test bottle in the Chemistry Department of the Experiment Station here and approve the suggestion of the Bureau of Standards that it be made the standard cream-test bottle.

Linwood A. Brown, Director, Department of Public Service Laboratories, Kentucky Agricultural Experiment Station.—Several years ago (1918) the State of Kentucky adopted by legislative enactment a standard milk and cream bottle, making it unlawful to use any other type of Babcock bottle in the purchase of milk or cream on the butterfat basis. The standard cream bottle is the same as recommended by the Bureau of Standards, namely, the 9 gram, 50 per cent cream bottle, but permitting the use of either the 6 inch or the 9 inch bottle. Very few concerns, however, use the 9 inch cream bottle, owing to the greater liability to breakage. As the result of 10 years' experience with the standard cream bottle, we can give our unqualified approval to the 9 gram, 50 per cent, 6 inch cream bottle, having found it uniformly accurate and generally satisfactory.

A. J. Gelpi, Instructor in Dairy Manufacture, Louisiana State University.—I would strongly recommend the adoption of one bottle for cream testing. The 9 gram, 50 per cent, short neck bottle is probably the most convenient and satisfactory one to use.

J. M. Bartlett, Chemist, Maine Agricultural Experiment Station.—I most certainly would welcome a standard bottle for this purpose, and after careful consideration I believe that the 9 gram, 50 per cent, short neck bottle would for all purposes be the most satisfactory. Of course one can read a little more accurately on a 9 inch bottle of the same capacity, but such bottles are much more difficult to handle, more easily broken and more difficult to wash out, and when a 9 gram sample is used the neck of the 6 inch bottle can be made small enough so the reading can be made accurately enough for all practical purposes.

R. C. Munkwitz, Assistant Professor in Dairying, University of Maryland.—It is as important to have uniform glassware in the analysis of dairy products as it is to have uniform containers for other products, if not more important. We are using a 9 gram, 50 per cent, short neck, 6 inch cream bottle as adopted by the Bureau of Standards and strongly recommend its adoption as the official bottle to be used by the Association of Official Agricultural Chemists.

Philip H. Smith, Official Chemist, Massachusetts Agricultural Experiment Station.—In reply to your letter of September 20, I feel that it is absolutely unnecessary to have three types of cream test bottles as standard in making butterfat determinations by the Babcock method.

T. H. Broughton, Director, Bureau of Dairying, Michigan Department of Agriculture.—We can see no particular objection to adopting the 9 gram, 50 per cent, 6 inch cream-test bottle. This would, of course, eliminate the 9 inch bottle now accepted by many

states including Michigan. There are very few of these bottles, however, and it is our opinion that you would find no particular objection to the elimination of this type on the part of the dairy concerns.

W. C. Geigler, Experiment Station Laboratory, Michigan Department of Agriculture.—We find upon reviewing our records that this type of bottle is the only one that is ever used in the testing of cream for milk fat in our laboratories. The Director of the Bureau of Dairying of the Department of Agriculture advises that this type of bottle is practically in universal use throughout Michigan. Only one concern uses any other type of bottle, and that firm uses the 9 gram, 50 per cent, 9 inch bottle. We believe there will be no difficulty in inducing the company to change over to the 6 inch bottle should the association make it the official standard bottle.

W. B. Combs, Professor of Dairy Husbandry, University of Minnesota.—A standard bottle should be selected for the Babcock cream test. It is further my opinion that the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle should be selected.

W. F. Hand, State Chemist, Mississippi Agricultural and Mechanical College.—We make very few determinations of fat in our laboratory by use of the Babcock method and we are not specialists, therefore, with regard to its details. For this reason we hesitate to express an opinion with reference to the adoption of one type of cream bottle. Since simplification is desirable on general grounds, we should feel inclined to favor the single type of bottle.

M. Gieger, Mississippi Experiment Station Laboratory.—We are using the 9 gram, 50 per cent, short neck, 6 inch bottles and like them better than any other.

A. C. Ragsdale, Chairman, Department of Dairy Husbandry, University of Missouri.—I think it would be our opinion that the Association of Official Agricultural Chemists in its official methods should adopt and recommend the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle inasmuch as that bottle has been adopted by the Bureau of Standards and is, I think, in general use.

H. P. Davis, Professor, Chairman, Department of Dairy Husbandry, University of Nebraska.—I believe that anything that can be done to encourage the use of a uniform bottle throughout the United States for the determination of fat in cream by the Babcock method is advisable.

J. M. Fuller, Professor of Dairy Husbandry, University of New Hampshire.—I am in favor of using the 9 gram, 50 per cent, 6 inch cream-test bottle. This is the size recommended by the American Dairy Science Association, and, as far as I know, is entirely satisfactory.

F. C. Button, Associate Professor of Dairy Husbandry, New Jersey Agricultural Experiment Station, New Brunswick, N. J.—Should advise that I feel such recommendation meets with our approval. In the work of Creamery Inspection in New Jersey for some years now we have used only the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle as the standard bottle in the state.

O. C. Cunningham, Professor, Department of Dairy Husbandry, New Mexico College of Agriculture and Mechanics Arts.—I consider this bottle to be the most practical cream-test bottle yet devised that is at the same time entirely satisfactory from the standpoint of accuracy.

H. C. Troy, Professor, Department of Dairy Industry, N. Y. State College of Agriculture.—Personally, I do not at present see why the Association of Official Agricultural Chemists should not continue to recommend the 50 per cent, 9 gram, long neck cream-test bottle. In my opinion it would be well not to recommend the 50 per cent, 18 gram, long neck cream-test bottle. * * * If only one cream-test bottle is recommended, I would prefer the 50 per cent, 9 gram, short neck bottle over either of the longer neck bottles.

T. H. Hopper, Agricultural Chemist, North Dakota Agricultural College.—I wish to

recommend that the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle be made the standard bottle of the association.

P. M. Brandt, Professor of Dairy Husbandry, Oregon State Agricultural College.—I will say that the use of the 9 gram, 50 per cent, short neck bottle meets with our hearty approval.

R. N. Brackett, Chief Chemist, Clemson College, South Carolina.—Though we have practically no work to do along this line, I would accept the Bureau of Standards selection.

W. H. MacIntire, Head, Department of Chemistry, University of Tennessee.—My grandma was a young lady when I last ran a Babcock test, and I therefore disclaim all claims to being qualified to pass an opinion. I do think, however, that it would be a good thing for the association to specify a single type of bottle, especially since this has been done by the Bureau of Standards. * * * I broached the matter to members of the staff of the Dairy Department and they expressed a decided preference for the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle.

G. S. Fraps, Chief, Division of Chemistry, Texas Experiment Station.—I think one bottle only should be adopted.

A. C. Merrill, In Charge, Dairy Manufacturing Department, Utah State Agricultural College.—We can see mainly one objection to adopting this suggested method, and that is that it would make it impossible for some plants to use the official methods where they have equipment for other types of tests, that is, tests other than the one the association adopts. On the other hand we believe it advisable because it would standardize the equipment for the official method for testing cream by the Babcock method, making results much more uniform. Believe for a test of this kind, where the gravimetric determination is made by measuring, the equipment should be standardized as well as the method of testing. * * * We believe it would be advisable to adopt the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle because it would conform with that adopted by the Bureau of Standards, and further because it is more universally used than any other style of bottle, therefore making the test for commercial purposes conform very closely with that for official purposes. It would have a tendency to discontinue the use of other style bottles which are, for obvious reasons, more undesirable for testing cream. Believe that a bottle could be made which would be much more accurate than this suggested bottle for official purposes, but we are depending upon the idea that the official method of running the Babcock test for cream will be made as practical as possible. Hope and urge the association to adopt a standard bottle and to adopt the type suggested, that is the one used by the Bureau of Standards.

A. W. Drinkhard, Jr., Director, Virginia Agricultural Experiment Station.—The workers at this institution use only one bottle for this purpose, which is the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle. We find no occasion to use a different type bottle and we believe it would be wise for the association to make this bottle the standard.

T. C. Johnson, Director, Virginia Truck Experiment Station.—I think it would be well to adopt the same kind of bottle that the Bureau of Standards has adopted for cream test purposes.

H. E. Bremer, Supervisor of Creamery Inspection, Vermont Department of Agriculture.—I believe it advisable to adopt one bottle as the standard bottle for testing cream and would suggest that the 9 gram, 50 per cent, 6 inch bottle be adopted. The graduations on this bottle to meet the requirements in Vermont would necessarily have to be divided into half per cents. In other words, we condemn cream bottles that are not graduated to the one-half per cent.

Charles P. Moat, Chemist, Vermont State Board of Health.—We have used the 9 gram, 50 per cent, short neck, 6 inch bottle for some time. If the A. O. A. C. wishes to use only one bottle, I think this is the bottle to use.

Burton G. Philbrick, Skinner, Sherman & Esselen, Inc., Research and Development Chemists, Boston, Mass.—We believe it would be a very good step. * * * We have ourselves standardized on the 9 gram, 50 per cent, short neck, 6 inch cream bottle, and believe that if this were adopted the minor disputes regarding the accuracy in calculating the different dimensions would be avoided, and the results obtained be as accurate as those obtained with many longer bottles or bottles of smaller capacity of neck.

Herbert E. Bowman, Milk Inspector, Somerville.—From my experience it would seem to me that the 9 gram, 50 per cent bottle is the most practical.

George E. Bolling, Milk Inspector, Brockton, Mass.—I would favor a bottle conforming with the standard adopted by the Bureau of Standards.

Frank E. Mott, Milk Inspector, Boston, Mass.—The 18 gram, 50 per cent, 6 inch bottle has long been obsolete, just as has also been the 6 inch, 10 per cent milk test bottle, and such bottles should no longer be recognized by the Official Association.

Forty persons, therefore, favor the adoption of a single type of bottle, and thirty-nine specify that this should be the 9 gram, 50 per cent, 6 inch cream-test bottle. Thirty-four of these forty people are officially connected with the law enforcement bodies and experiment stations of this country and represent thirty different States.

The following persons may be said to be "on the fence":

Walter R. Freeman, State Dairy Commissioner, Colorado.—For our conditions in Colorado, the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle would be very satisfactory. This especially in the country-buying stations. * * * However, we have found in the case of direct shippers and larger producers in the country that their cream often runs higher than 50 per cent. Our creameries in the state are gradually making use of the 55 per cent bottle, instead of the 50 per cent. For this reason, I would rather see it adopted than the 50 per cent bottle, as it would not necessitate the operator's splitting the samples. This bottle is made similar to the 50 per cent, 6 inch bottle with the exception that the neck is often $6\frac{1}{2}$ inches instead of 6 inches.

C. S. Robinson, Chemist, Michigan Agricultural Experiment Station.—This laboratory does no testing of this character and consequently, from experience, I have no notion of the advisability of the adoption of a standard bottle. On general principles, however, the suggestion seemed to be a good one and it would also appear to be desirable to have the same bottle which has been adopted by the Bureau of Standards.

Charles D. Howard, Chemist, New Hampshire State Board of Health.—While I am not anxious for standardization on the 9 gram bottle, I would not object to it.

W. D. Swope, Associate Professor of Dairy Husbandry, Pennsylvania State College.—It seems very desirable to have but one type of bottle for the determination of fat in cream by the Babcock method. At the present time, I believe the 9 gram, 50 per cent, 6 inch cream-test bottle is most desirable. I believe that it would be better for us to eventually adopt a bottle with a longer neck and less diameter so that we could overcome the error which we often have in reading our present 9 gram, 50 per cent bottles. It would probably take some time to bring about this change, but by making a longer necked bottle, also legal, the change could be made gradually, especially as our old machines wear out and in this way work little hardship upon any plant or individual. As to the length of neck that would seem most desirable, some little thought should be given to it and possibly some experimental work conducted.

James W. Kellogg, Director, Bureau of Foods and Chemistry, Pennsylvania Department of Agriculture.—* * * We are making legal analyses, therefore, from the standpoint of the specifications laid down by the Pennsylvania Bureau of Standards, as well as the A. O. A. C., if we employ either one of the above types of bottles. We have

preferred and are using the 50 per cent, short neck, cream-test bottle in our work and could very readily adhere only to this type, which it is suggested, according to your letter, the A. O. A. C. adopt as the standard bottle of the association.

J. L. St. John, State Chemist, Washington Experiment Station.—I should, in general, favor the use of one type of bottle for the fat determination in cream. We have not, however, in this laboratory, run a sufficiently large number of such determinations to be able to express a very decided preference in this matter, and, also, I do not feel that we would be able to express a definite preference for the type of bottle which might be best suited for this use.

L. E. Waller, State Chemist, Wyoming.—I believe that the Association of Official Agricultural Chemists might well adopt such a bottle but only after thorough study of the matter in question.

In this class there are seven persons representing six departments in six states who are more or less favorable to the single type of bottles, but do not absolutely prefer the 9 gram, 50 per cent, 6 inch cream bottle. The rules of the association should be tested by means of their exceptions and the objections to the proposed change should be carefully scrutinized. The persons who object are men of consequence in their communities, and their opinions should be respected.

C. L. Roadhouse, Head, Division of Dairy Industry, University of California.—In conference with Mr. D. H. Nelson of this Division, who has charge of our Dairy Testing work, I have considered this question, and we are in full accord that you would be making a mistake to recommend the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle. The reason for our opinion is as follows: The distance between the smallest graduations of the 6 inch bottle is so small that it allows a comparatively large error in reading. With a longer necked bottle these smallest graduations would be farther apart, making it easier to obtain an accurate reading. In California our State Dairy Law permits the use of two types of cream-test bottles, the long neck, 9 inch, 50 per cent, 9 gram bottle, and the long neck, 9 inch, 50 per cent, 18 gram bottle. The graduated portion of the neck shall be not less than 120 mm.; the smallest graduation shall represent 0.5 per cent. The only condition under which we would justify the 6 inch bottle is where the centrifuge is not constructed to accommodate the 9 inch bottle. We believe that for all official purposes such centrifuges should be provided.

E. M. Bailey, Chemist in Charge, Analytical Laboratory, Connecticut Agricultural Experiment Station.—The present specifications for apparatus and chemicals for testing milk and cream as given in our association methods are the result of a joint conference between representatives of the A. O. A. C. and the American Dairy Science Association. I do not recall whether the American Public Health Association was represented or not, but at any rate they have accepted these specifications and the A. O. A. C. procedure for testing so that there is now pretty general uniformity among all operators in carrying out the technic of milk and cream testing, and such uniformity is important and highly desirable. It was agreed at this conference that the specification for Babcock glassware as laid down by the U. S. Bureau of Standards should be accepted as official by these associations. If now the Bureau of Standards recognizes only one standard cream-test bottle, I think we might well consider deleting the other two types which we now recognize. But it is by all means advisable to have assurance that the Dairy Science Association and the Public Health Association will take similar action so that these three organizations will continue to be in agreement as they are at present. In this State the 50 per cent, 9 gram, short neck cream-test bottle is used almost exclusively. We rarely ever see the long neck type. It may be, however, that the latter serve some need among our association members and others, and ought to be retained.

H. H. Hanson, State Chemist, Delaware State Board of Agriculture.—The writer has used various kinds of cream-test bottles during the last 27 years, and is inclined to personally favor a small neck bottle, believing that a more accurate test can be obtained with it. Years ago, while at the Maine Experiment Station, we used the so-called Bartlett cream-test bottle, which contained in the middle of the stem a bulb, similar to the bottles now used for butterfat test, and known as the Hortvet butterfat test bottle. With this type, there were graduations both below and above the bulb, and very close and accurate readings could be made. I suppose this bottle as a standard is now out of the question. Personally, I do not like the 50 per cent cream bottles, preferring to use a 30 per cent bottle of the present common style, taking a 9 gram charge for such creams as run more than 30 per cent, but if we must confine ourselves to a 50 per cent bottle, I see no particular object in limiting our standard to one size bottle. I should prefer to allow our present A. O. A. C. standards to remain as they are, and give the analyst a choice of three bottles. Our State law standardizes the cream-test bottles allowed, according to the A. O. A. C. standards.

J. H. Skinner, Director, Indiana Agricultural Experiment Station.—* * * I am, therefore, sending you a copy of the letter from Professor Gregory, with a copy of the statement we use in this State. I believe this answers your question in general, and since different States may have different laws on this subject, it would seem to me that the Association of Official Agricultural Chemists in its official methods should go slowly in recommending any one type of bottle for use in determining butterfat in cream by the Babcock method.

H. W. Gregory, Chief of Dairy Husbandry, Indiana Agricultural Experiment Station.—In Indiana we have always permitted the use of either a 6 inch cream-test bottle or a 9 inch cream-test bottle, but these two types of bottle must comply with the following specifications, which are attached. Most of the creameries use the 6 inch bottle, but there are a few that do the testing in their plants that use the 9 gram, 50 per cent, long neck bottle. However, we would much prefer the short neck, 50 per cent, 9 gram bottle.

The specifications for the Indiana standard cream-test bottles include the 50 per cent, 9 gram, short neck bottle and the 50 per cent, 9 gram, long neck bottle.

Wyatt W. Randall, Chief, Bureau of Chemistry, Maryland Department of Health.—I do not approve of the suggestion, because: (1) I believe the 9 gram, 50 per cent, 9 inch bottle is used to some extent, and, as it is capable of being read with far greater accuracy than the 6 inch, I see no reason why it should be forbidden those who prefer it and have centrifuges adapted to its use. I see no sense in displacing a more accurate piece of apparatus by means of a less accurate one, merely to secure uniformity. (2) I have always maintained that a cream-test bottle whose graduations are only $\frac{2}{3}$ mm. apart, while the size of the neck is such that 65 mm. of it has a capacity of 5 cc., is an ill-chosen "official bottle". Very few samples of cream ever furnish a fat column more than half the length of the graduated part of the neck. I should hate to feel that the only official cream-test bottle we had was the one listed as "(1)" in "Standard Methods". Bottles "(2)" and "(3)" have at least the value of being employable with an approach to accuracy.

S. C. Dinsmore, State Food and Drug Commissioner, Nevada.—The use of the 9 gram, 50 per cent, 6 inch, cream-test bottle is illegal in this state. We prefer the 9 inch, 9 gram bottle for our work.

Guy G. Frary, State Chemist, South Dakota.—In my limited experience it has seemed to me that the 9 gram, 50 per cent, long neck, 9 inch bottle is preferable to the 6 inch bottle. The longer neck, of course, makes possible greater distance between the gradua-

tions and hence tends to more easy and accurate reading of the test. I recognize that it is quite possible that workers at the Bureau of Standards or elsewhere have detected errors in the 9 inch bottle which may not exist in the 6 inch bottle, and it is possible, of course, that it has been found that the 6 inch bottle gives just as accurate or more accurate readings than the longer instrument. However, unless such facts have been determined, I would be inclined to continue in the official methods at least the two bottles, 9 gram, 50 per cent, long neck, 9 inch bottle and the 9 gram, 50 per cent, short neck, 6 inch bottle. If we are to go to one bottle exclusively, then I would prefer, with the knowledge that I now have, that the 9 gram, 50 per cent, long neck, 9 inch test bottle be made the standard.

H. E. Jackson, Professor of Dairy Husbandry, University of Wisconsin.—The dairy laws of this state do not recognize a 9 gram cream-test bottle, but state that the unit for testing shall be 18 grams and that a 30, 40 or 50 per cent bottle may be used. As long as this law remains in force in this state, it of course would not be possible for a standard 9 gram bottle to be used at this place. It is possible that other states have rulings of this kind, although the writer has not looked up the laws concerning this. This of course does not necessarily mean that the Association of Official Agricultural Chemists should not recommend such a bottle in its official methods. It would seem that if such a recommendation is made, namely, that a specific type of bottle should be used, it should be done with the knowledge or understanding that this type of bottle is more accurate than any other type. So far as the writer knows, this Department has conducted no experiments to determine whether the 9 gram, 50 per cent, short neck, 6 inch cream-test bottle is a better bottle to use than the 9 inch bottle using the 18 gram charge. The writer personally prefers the 9 gram bottle with a 9 inch neck, but has never conducted any experiments to prove whether or not this bottle is more accurate than the others. Unless there has been considerable work done in checking these various types of bottles, it would not seem to be a good plan to recommend a definite bottle.

This represents nine official chemists in opposition, residing in eight States. If to these nine are added the seven who are "on the fence", and the one who is noncommittal as to the type of bottle he would like to see adopted, is included, there are seventeen official chemists in State employ not absolutely in favor of the change as compared with thirty-four such chemists in absolute favor of the change.

Under these circumstances, the referee recommends¹ that there be deleted from *Methods of Analyses*, Chap. XIX, Sec. 37, the description of the 9 gram, long neck, 9 inch, 50 per cent, cream-test bottle and the 50 per cent, 80 gram, long neck, 9 inch, cream-test bottle.

REPORT ON MILK.

By HENRY HOFFMANN, JR. (State Agriculture, Dairy and Food Dept., St. Paul, Minn.), *Associate Referee*.

The work done on milk this year was confined to a study of a method for the preparation of standard pads for collecting the visible dirt in routine milk samples for the purpose of comparison or classification

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 67 (1930)

and of the method for the determination of specific gravity of milk by means of a hydrometer, with the purpose of standardizing the lactometer and including definite directions or instructions for its use.

After studying various methods for testing milk for visible dirt, the associate referee proposes a method for the preparation of standard pads. This method, which makes use of a 50 per cent cane sugar solution as the suspending medium of the dried stable dirt, was tried out by seven collaborators.

DETERMINATION OF SEDIMENT IN MILK.

As "clean" milk is greatly to be preferred to "cleaned" milk, many food control officials object to the use of the sediment test; however, with proper dairy inspection, this test is a valuable means of securing clean milk. Since it is practically valueless if the milk has been thoroughly strained or clarified, it is of greatest value when used at receiving and pasteurization plants.

Two methods for ascertaining cleanliness of milk have been proposed or used in the past. The centrifugal method is probably the older of the two methods. The result is reported by volume of sediment obtained from a definite quantity of milk in a graduated tube, on the wet basis. The other method, which has found much more favor among the food and dairy control chemists and others, is the filtration method. A specified volume of milk is filtered through a disc of absorbent cotton free of starch or sizing, and the amount of discoloration and filth or sediment deposited on the pad is noted; the quantity of milk ordinarily used is one pint.

In order to make comparisons between results reported by different analysts, a set of standard gages are necessary, and it is proposed to prepare a set that will enable analysts to compare their routine milk pads. The American Public Health Association¹ shows a series of photographs of pads containing definite amounts of sediment prepared independently by three different collaborators. The photographs show a similarity among the pads containing various amounts of sediment. These pads should be prepared according to a definite method, then disinfected and preserved.

SEDIMENT TESTERS.

Of the several types of apparatus on the market for determining sediment in milk, probably one of the most highly recommended is that known as the Wisconsin Sediment Tester. In this tester the filtering of one pint, which is the usual quantity, requires only a few seconds. The milk is forced through a screen in a cylinder that tapers at the bottom to an opening of 1 inch in diameter. The fixed disk of absorbent

¹ *Standard Methods Milk Analysis*, 5th ed. (1927).

cotton is placed in the cap at the bottom and the sediment is collected on this disk. As the diameter of the opening is 1 inch, the sediment is spread over a circle exactly one inch in diameter.

One of the other testers in common use is the Wizard Sediment Tester. The cotton disk is fitted in the device, which is then placed over a full pint bottle sample of the milk to be tested. The full bottle is next inverted over an empty bottle. By squeezing the bulb the pressure causes the milk to pass through the disk, which retains all dirt. The screen disk in this apparatus is also one inch in diameter, but the actual opening for the milk to flow through is approximately seven-eighths of an inch in diameter. The Vacuum Sediment Tester also has a disk on which the sediment in the milk is spread over a circle exactly 1 inch in diameter. There is considerable variation in the ease with which different samples or lots of milk can be filtered. Often the warming of the sample of milk will aid the process of filtering. The pasteurized milk causes trouble more often than the raw milk. This seems to be due to a physical change in the fat and also to a partial coagulation of the albumen by the high temperatures used in pasteurization.

If more than one type of tester is used in a laboratory for the control work of inspecting the amount of sediment in market milks and these types produce a sediment pad of a different diameter, a set of standards should be prepared for each type of tester used. Pint samples of milk only are regarded as standard. If a greater or less quantity of milk is used, reports must state the exact size of the sample used. If the settlings or the bottom milk of a can is used in making the test, this fact must be stated in the report.

PREPARATION OF STANDARD SEDIMENT PADS.

A suspension of weathered, dried, finely ground cow dung is first prepared, a 50 per cent cane sugar solution being used as the medium. The cow dung is dried in an oven and ground through a laboratory feed mill several times. Practically all the ground material should be less than 60-mesh fineness, and the greater portion should be finer than 100 mesh. One-tenth gram is accurately weighed and transferred to a 1000 ml. measuring flask, the 50 per cent sugar solution also being used to wash all the fine particles down into the flask. The volume is made up to the 1000 ml. mark with more 50 per cent sugar solution after most of the fine particles have been wetted by shaking the half-filled flask thoroughly several times. After the volume is made up to the mark, the contents of the flask are shaken vigorously every 5 minutes for sufficient time to saturate the particles thoroughly (one-half hour to one hour). When the particles have been thoroughly wetted it will be noted that the sugar solution will hold them very evenly in suspension, and the mixture is now ready to use in making the standard pads.

On the basis of 0.1 gram per 1000 ml., 10 ml. of the sugar solution contains 1 mg. of sediment. Test pads are made with one of the sediment testers described, varying volumes of the sediment solution being used. Several ounces of filtered skimmed milk are placed in the sediment tester and varying volumes of the sediment solution are added. After forcing the milk through the pad, a small quantity of filtered skimmed milk is run through. By using a small quantity of milk in the tester at the time the sediment solution is added, a more even distribution of the sediment on the pad is obtained. The purpose of following through with more skimmed milk is to be sure that all the fine particles are washed onto the pad and also to obtain a casein or adhesive condition so that the sediment will adhere to the pad. Adding the sediment sugar solution directly to a small volume of milk and filtering the entire amount does away with a possibility of any error entering into the results due to poor sampling. The pad is then removed from the tester, mounted permanently on a stiff paper, allowed to dry, and then made permanent by spraying with a strong disinfectant such as corrosive sublimate. A good apparatus for this purpose is an ordinary throat atomizer, provided caution is observed not to use corrosive sublimate in contact with metal. Below each mounted standard pad on the paper should be noted the quantity of dried material that the dirt or filth on the pad represents.

CAUTIONS.

The following directions were sent to the collaborators:

- (1) Be sure to obtain well weathered cow dung; if this is not possible, the associate referee will send you some.
- (2) Before measuring out each aliquot portion, shake the suspension well.
- (3) Prepare pads within a few hours after suspension has been made.
- (4) When sending the pads, use a small box so that they will be well protected in the mail.

Each collaborator is to prepare a series of mounted standard pads representing 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 mg. of the dried dung. It is a good plan to mount a perfectly clean pad for comparison. Below each pad the amount of sediment it represents should be noted and also any criticisms or information as to how that amount of sediment, if found in a pint sample of market milk, would be classed. The purpose is to learn what standard the average food control chemist is using.

COMMENTS ON RESULTS.

The pads prepared by the different collaborators represent sediment varying from 0.1 to 10 mg. The results obtained in all cases were encouraging and showed a relative increase of sediment in each pad. The cotton disks prepared by the different collaborators and representing the same amount of visible dirt showed very similar results. The associate referee at this time wishes to thank the following collaborators for

their interest in this matter: F. L. Mickle, W. R. Richardson, Thos. A. Buckland, J. C. Marquardt, A. H. Robertson, G. A. Dysterheft and M. A. Goodwin.

SPECIFIC GRAVITY.

The tentative method for determining the specific gravity of milk given in *Methods of Analysis*, A. O. A. C., reads as follows:

Determine specific gravity at 15.6°/15.6°C. (60°/60°F.) by means of a pycnometer (cf. p. 361, 3), or by means of a standardized hydrometer.

It was noted in a review of the various text books and the literature for the directions of reading the lactometer that some of the authors advise reading the instrument on the same level as the surface of the milk, that is at the bottom of the meniscus, and that others are somewhat indefinite.

In direct opposition to this practice the manufacturers at the present time are calibrating these instruments to be read at the top of the meniscus, and it is upon this practice that the U. S. Bureau of Standards issues certificates on lactometers. Then, too, actual experience and observation show that there are various types and sizes of lactometers on the market. Some of these are designed to enable the operator to obtain a fairly accurate reading of the specific gravity of milk, while a large percentage is inaccurate and too small for practical use.

RECOMMENDATIONS¹.

It is recommended—

(1) That the amount of sediment in milk be reported as visible dirt corresponding to — mg. of desiccated cow manure per pint or other quantities.

(2) That the proposed method for the preparation of the standard pads of cotton disks containing a definite amount of desiccated cow manure with which the sediment pads of routine milk samples can be compared be adopted as a tentative method.

(3) That the method for the determination of the specific gravity of milk by means of the hydrometer be studied with the object in view of including specifications of the lactometer and instructions for reading same in the method.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 67 (1930).

REPORT ON BUTTER.

By HENRY HOFFMANN, JR. (State Agriculture, Dairy and Food Dept., St. Paul, Minn.), *Associate Referee*.

Although it was not possible during the current year for the Associate Referee on Butter to arrange collaborative work, the use of tin containers for butter samples in place of glass containers was studied.

In the official method for the preparation of a butter sample the softening of the entire sample in a closed vessel at as low a temperature as possible is specified. The sample is then shaken vigorously until a perfectly homogeneous semi-solid mass is obtained and portions for analysis are weighed out. The advantages of using a glass container are apparent. The appearance of the butter will indicate to the experienced analyst when it is sufficiently soft to be mixed properly, and it is not always necessary to use a bath with a thermometer.

In 1927 L. C. Mitchell¹ attempted to define this condition by means of a thermometer. He found that some samples had to be stirred at 34°–35°C. to obtain the proper consistency, while others were far too fluid at 33°C. and had to be stirred at 31°–32°C. As no definite degree of temperature for each sample can be given, it is necessary for the analyst to have the sample continuously under his direct observation. Then, too, there is the danger of loss of moisture when the container is opened for examination. An analyst might open a tin container several times if he were in a hurry to prepare a sample for analysis, because extra time and work are necessary when a sample of butter has been overheated and separation has occurred.

If the metal containers are not built of heavy-weight material, they lose their shape, the fit of the covers is questioned, or a loss of moisture or of a portion of sample might occur. The wear which tin containers receive is severe, owing to the brine, and within a short time the danger of contamination of the butter with rust would arise.

The only advantage of a metal container over a glass one is that of decrease of breakage. This advantage, however, is outweighed by the disadvantages.

RECOMMENDATIONS².

It is recommended—

(1) That the recommendation adopted in 1927 calling for further study of the use of metal containers for butter samples be discontinued.

(2) That the other studies recommended in 1927 be continued.

¹ *This Journal*, 11, 267 (1928).

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 67 (1930).

REPORT ON CHEESE.

By ERWIN O. HUEBNER (Department of Agriculture and Markets,
Madison, Wis.), *Associate Referee*.

During the past year an effort was made to find a satisfactory method for determining lactose in process cheese and cheese compounds.

The presence of lactose or sucrose in process cheese and cheese compounds is the result of the addition of such sugars during manufacture. Lactose may be added in the form of milk, condensed milk, cream or concentrated whey. In a few days, however, through fermentation the lactose changes to lactic acid.

Since the quantity of lactose in process cheese products found on the market was found to be as low as 1 per cent, a polarimetric method for its estimation proved to be inapplicable. In general the method of clarification used in the tentative quantitative method for the determination of citric acid¹ was followed in the procedure outlined below.

LACTOSE.

Quantitative Method.

REAGENTS.

- (a) *Sodium oxalate solution*.—Dissolve 2 grams of sodium oxalate in 100 cc. of water.
- (b) *Sulfuric acid solution (1 + 1)*.
- (c) *Phosphotungstic acid solution*.—Dissolve 20 grams of phosphotungstic acid in water and dilute to 100 cc.
- (d) *Finely powdered anhydrous sodium sulfate*.
- (e) *Solid potassium chloride*.

DETERMINATION.

Weigh 25 grams of the ground cheese into a 500 cc. wide-mouthed salt bottle, and add, in 25 cc. portions, 100 cc. of water at a temperature of 50°–60°C., shaking vigorously after each addition. If necessary, continue the shaking until the cheese is thoroughly broken up. Add 25 cc. of the sodium oxalate solution and shake vigorously for 1 minute; add 25 grams of the powdered sodium sulfate and shake for 2 minutes; add 10 cc. of the sulfuric acid solution and shake; and then add 25 cc. of the phosphotungstic acid solution and shake vigorously. Transfer the contents of the bottle to a 300 cc. volumetric flask, cool immediately to 20°C., and make to the mark with water. Mix thoroughly, allow to stand for 10 minutes, and then filter through a dry folded filter. Transfer 100 cc. of the filtrate to each of two 200 cc. volumetric flasks, add 10 per cent sodium hydroxide solution to one flask until the mixture is alkaline to litmus, then add 5 grams of solid potassium chloride, and mix thoroughly. Cool to 20°C. and make to the mark with water. Shake well, allow to stand for 10 minutes, and filter through a dry folded filter. Determine the lactose in a 50 cc. aliquot, as directed on p. 195, 51, of *Methods of Analysis*, A. O. A. C. Treat the contents of the other volumetric flask as directed on p. 187, 23(C), add 10 per cent sodium hydroxide solution until alkaline to litmus, and add 5 grams of solid potassium chloride. Mix thoroughly, cool to 20°C., and make to the mark with water. Let stand for 10 minutes.

¹ *This Journal*, 11, 41 (1928).

Determine the lactose in a 50 cc. aliquot as before. An agreement between the amount of cuprous oxide reduced before and after inversion establishes the absence of sucrose.

Since the insoluble material of cheese and the precipitated phosphotungstic acid occupies some space in the flask as originally made up, it is necessary to correct for this volume. From the average composition of cheese the volume of the precipitate was calculated to be 14 cc. To obtain the true amount of lactose present, all results must be multiplied by the factor 0.95.

Known amounts of lactose were added to several samples of cheese. These were analyzed according to the procedure outlined above. The quantities added and the quantities found are given in Table 1.

TABLE 1.
Recovery of lactose added to cheese.

| QUANTITY ADDED | QUANTITY FOUND |
|-----------------|-----------------|
| <i>per cent</i> | <i>per cent</i> |
| 0.83 | 0.78 |
| 1.66 | 1.68 |
| 2.49 | 2.43 |
| 3.33 | 3.35 |
| 4.44 | 4.40 |

These results indicate that the recovery of lactose is satisfactory and within the range likely to be found in process cheese products.

Since sucrose has been used in the past in the manufacture of process cheese it was deemed of interest to determine whether that sugar could be recovered quantitatively when added to cheese samples. The procedure was repeated, sucrose being substituted for lactose. As the following table shows, the recovery was as satisfactory as when lactose was used.

TABLE 2.
Recovery of sucrose added to cheese.

| QUANTITY ADDED | QUANTITY FOUND |
|-----------------|-----------------|
| <i>per cent</i> | <i>per cent</i> |
| 0.83 | 0.78 |
| 1.67 | 1.63 |
| 2.05 | 1.96 |
| 2.49 | 2.44 |
| 3.28 | 3.32 |

The reducing sugars before and after inversion were calculated to invert sugar. The percentage of invert sugar before inversion was subtracted from the percentage of invert sugar after inversion and the difference was multiplied by 0.95 to obtain the percentage of sucrose.

The procedure was devised primarily for lactose, as that is the sugar found in many of the process cheese compounds that are on the market under coined names, but as shown by the results in Table 2 it can also

be used to determine sucrose quantitatively when that sugar is freshly added to cheese. No work was attempted on mixtures of these sugars in cheese. It is a question for further study¹.

REPORT ON DRIED MILK.

By E. L. P. TREUTHARDT (Food, Drug and Insecticide Administration, Boston, Mass.), *Associate Referee*.

The work on dried milk followed the recommendation approved at the October, 1928, meeting that the associate referee study methods for the determination of total fat in dried milk.

The present tentative method² is an adaptation of the Roesse-Gottlieb method. When used on whole milk powders, low results are sometimes obtained, which Keister³, the previous referee, ascribes to imperfect details in the preparation of the sample for extraction.

Preliminary work included fat determinations on two samples by the tentative method and by the following modification taken from the methods of the American Dry Milk Institute:

After the first extraction with ethers, add 4 cc. of 95 per cent alcohol to the liquid remaining in the extraction apparatus, mix, then proceed with the second extraction. In the third extraction add, if necessary, sufficient water to raise the level of the aqueous layer to its original volume.

The results are given under "Tentative Method" and "Modified Tentative Method" in Table 1. Considerable difficulty was encountered by the formation of emulsions when the tentative method was used. The purity of the fats was checked, and no correction was found to be necessary.

TABLE 1.

Collaborative results on determination of fat.

| SAMPLE | ANALYST | TENTATIVE METHOD | MODIFIED TENTATIVE METHOD | ALIQOT METHOD | ALIQOT WITH 2ND ALCOHOL |
|--------|---------------------|---------------------|---------------------------------|------------------|-------------------------------|
| | | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| A | E. L. P. Treuthardt | 26.75 | 26.85 | 27.00 | 26.20 |
| | | 26.50 | 26.85 | 26.17 | 25.53 |
| | H. W. Haynes | 26.66 | 26.53 | | |
| | | | 27.05 | | |
| | C. H. Hickey | 25.80 | 26.85 | 18.75 | 23.00 |
| | | 26.50 | 26.70 | 18.15 | 18.35 |
| | W. M. Goldberg | 26.24 | | | 26.21 |
| | | 26.59 | | | 27.18 |
| | | 26.65 | | | |
| | B Treuthardt | 28.30 | 28.40 | | |
| | | 27.75 | 28.40 | | |

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 67 (1930)

² *This Journal*, 8, 482 (1925).

³ *Ibid.*, 10, 308 (1927).

Collaborative results on Sample No. 1.

(Expressed in percentage.)

| COLLABORATOR | MOISTURE | TENTATIVE METHOD | | MODIFIED METHOD | | TENTATIVE METHOD WITHOUT FILTER | | MODIFIED METHOD WITHOUT FILTER | |
|------------------------------------|--------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|---|----------------------------------|--|----------------------------------|
| | | Fat | On Dry Basis | Fat | On Dry Basis | Fat | On Dry Basis | Fat | On Dry Basis |
| L. A. Salinger San Francisco | 4.25 | | | | | 21.34 22.20 23.19 23.93 23.58 | 22.29 23.19 24.99 24.63 | 25.85 26.00 26.72 25.91 | 27.00 27.10 26.72 27.06 |
| L. W. Ferris Buffalo | 3.87 | 23.94 24.14 | 24.91 25.11 | 26.26 26.30 | 27.32 27.36 | 24.24 24.49 | 25.22 25.48 | 26.62 26.26 | 27.69 27.32 |
| J. I. Perlman Albany | 4.28 | 25.79 25.68 | 26.94 26.83 | 26.42 26.46 | 27.60 27.64 | 25.65 25.58 (1) 25.74 (1) 25.63 | 26.80 26.72 26.89 26.78 | 26.63 26.72 (1) 26.65 (1) 26.47 | 27.82 27.92 27.84 27.65 |
| H. Rogavitz (4) New York | 3.82 3.70 | 19.70 20.03 | 20.48 20.88 | 24.87 25.02 | 25.86 26.01 | 22.09 21.22 | 22.94 22.04 | 25.26 25.49 | 26.23 26.47 |
| W. M. Goldberg (4) New York | 4.23 3.87 | 25.33 21.70 | 26.45 22.66 | 26.60 23.99 | 27.78 25.05 | 25.84 24.74 | 26.88 25.74 | 26.33 26.47 | 27.39 27.54 |
| C. B. Stone Minneapolis (5) | (2) 3.95 | 22.79 22.91 22.72 23.93 | 23.69 23.81 23.68 24.94 | 22.71 23.31 25.44 25.29 | 23.64 24.26 26.52 26.36 | 22.30 24.61 | 23.22 25.62 | 24.96 25.08 | 25.95 26.08 |
| M. J. Gnagy (3) Minneapolis (5) | (2) 3.85 | 25.39 24.83 25.61 26.15 | 26.35 25.76 26.65 27.21 | 19.62 23.75 25.67 25.58 | 20.40 24.70 26.72 26.69 | 22.40 26.10 | 23.30 27.15 | 25.92 25.91 | 26.98 26.97 |
| E. C. Thompson New York | 4.26 | 25.60 25.65 | 26.17 26.79 | 26.13 26.54 | 27.29 27.72 | 26.31 26.18 | 27.48 27.35 | 26.45 26.45 | 27.63 27.63 |
| H. A. Reed Seattle | 4.07 | 25.05 24.16 24.63 | 26.11 25.19 25.68 | 25.48 25.57 25.80 25.71 | 26.56 26.66 26.90 26.80 | 25.33 25.40 | 26.41 26.48 | 26.56 26.50 26.52 26.46 | 27.69 27.63 27.65 27.58 |
| P. L. Leavitt Boston | 3.97 | 23.85 24.93 | 24.84 25.96 | 25.99 26.36 | 27.06 27.45 | 24.41 23.98 | 25.42 24.97 | 26.37 26.31 | 27.46 27.40 |

| | | | | | | | | | |
|-------------------------------|------|---|----------------------------------|----------------------------------|----------------------------------|---|---|----------------------------------|----------------------------------|
| H. W. Haynes Boston | 3.77 | 25.73 25.64 | 26.74 26.64 | 26.78 26.49 | 27.83 27.53 | 26.72 24.97 25.98 26.07 24.60 | 27.77 25.95 27.00 27.09 25.56 | 26.27 26.03 | 27.30 27.05 |
| C. H. Hickey Boston | 3.98 | 24.10 24.05 | 25.10 25.05 | 25.50 24.80 26.00 26.50 | 26.56 25.83 27.08 27.60 | 24.10 24.40 | 25.10 25.41 | 25.25 26.60 26.20 26.00 | 26.30 27.70 27.29 27.08 |
| E. L. P. Treuthardt Boston | 3.83 | 25.13 24.76 | 26.13 25.75 | 26.25 26.25 | 27.30 27.30 | 24.82 | 25.81 | 26.40 26.10 | 27.45 27.14 |
| D. Dahle Savannah | 5.27 | 23.69 24.49 | 25.01 25.86 | 25.47 25.53 | 26.89 26.96 | | | | |
| J. T. Keister Washington | 4.01 | 25.73 25.76 25.84 | 26.80 26.83 26.92 | 26.53 26.49 | 27.63 27.59 | | | | |
| R. S. Pruitt St. Louis | 4.55 | 24.11 24.16 | 25.26 25.31 | 26.15 26.48 | 27.40 27.74 | | | | |
| F. Hillig Washington | 4.08 | 25.26 25.19 | 26.34 26.26 | 25.55 25.38 | 26.64 26.46 | | | | |
| T. C. Dunn Chicago | 3.68 | 24.91 24.76 | 25.87 25.71 | 25.83 25.75 | 26.82 26.74 | | | | |
| W. J. McCarthy Cincinnati | 3.90 | 21.85 21.70 | 22.74 22.58 | | | | | | |
| G. A. Ayer (6) Chicago | | 26.82 25.55 26.45 25.85 25.45 25.07 25.47 25.46 25.41 | 26.92 25.87 26.76 25.70 | 26.50 25.52 26.02 26.15 | 26.50 25.87 26.76 25.70 | 24.82 24.32 24.71 26.13 | 25.75 26.05 | 26.11 26.90 26.76 27.64 | |
| O. Haydon (6) Chicago | | 25.47 26.82 19.70 | 25.33 27.21 20.48 | 25.60 26.92 19.62 | 26.62 27.83 20.40 | 24.73 26.72 21.22 | 25.55 27.77 22.04 | 26.18 27.24 24.96 | 27.23 27.92 25.95 |
| Average Maximum Minimum | | | | | | | | | |

Notes: (1) Aluminum dish for weighing fat.
 (2) Average.
 (3) 4-6 extractions made.
 (4) Rogavitz and Goldberg analyzed same subdivision.
 (5) Stone and Gaagy
 (6) Ayer and Haydon

| | | | | | | | | | |
|---------------------|------|--|-------------------------|--|----------------------------------|--|----------------------------------|--|----------------------------------|
| P. L. Leavitt | 4.53 | 23.62 23.72 | 24.74 24.85 | 23.92 23.99 | 25.06 25.13 | 23.53 23.79 | 24.65 24.92 | 23.69 23.71 | 24.81 24.84 |
| H. W. Haynes | 3.78 | 23.84 23.70 (4) 23.80 | 24.78 24.63 24.94 | 23.80 23.88 (4) 24.03 (4) 23.94 | 24.74 24.82 25.17 25.08 | 23.55 23.85 (4) 23.76 (4) 23.70 | 24.48 24.79 24.89 24.82 | 23.60 23.45 (4) 23.60 (4) 23.60 | 24.53 24.37 24.95 24.72 |
| C. H. Hickey | 4.92 | 23.75 23.90 | 24.98 25.14 | 23.60 23.25 | 24.82 24.45 | 23.60 22.90 | 24.82 24.08 | 23.15 23.20 | 24.35 24.40 |
| E. L. P. Treuthardt | 4.71 | 23.91 23.30 | 25.09 24.45 | 24.00 | 25.19 | 24.00 23.93 | 25.19 25.11 | 23.97 23.95 | 25.16 25.14 |
| D. Dable | 6.21 | 23.26 23.28 | 24.80 24.82 | 23.17 23.24 | 24.71 24.78 | | | | |
| J. T. Keister | 4.94 | 23.66 23.86 | 24.88 25.09 | 23.80 23.78 | 25.04 25.01 | | | | |
| R. S. Pruitt | 5.59 | 23.28 23.29 | 24.66 24.67 | 23.65 23.38 | 25.05 24.76 | | | | |
| F. Hillig | 4.97 | 23.31 23.54 | 24.53 24.77 | 23.55 23.36 | 24.78 24.58 | | | 23.92 24.02 23.52 23.97 | |
| T. C. Dunn | 4.69 | 23.05 23.13 | 24.19 24.27 | 23.46 23.40 | 24.62 24.56 | | | | |
| W. J. McCarthy | 4.87 | 23.40 23.32 | 24.60 24.52 | | | | | | |
| G. A. Ayer (7) | | 23.22 23.21 25.10 23.45 24.35 22.64 | | 23.97 23.12 | | 24.14 24.10 | | | |
| O. Haydon (7) | | 23.02 22.84 | | 23.61 23.58 | | 24.34 24.97 | | 24.70 25.32 23.46 23.83 | |
| Average | | 23.47 | 24.68 | 23.51 | 24.73 | 23.64 | 24.78 | 23.76 | 24.90 |
| Maximum | | 25.10 | 25.34 | 22.03 | 25.21 | 24.97 | 26.27 | 25.32 | 26.54 |
| Minimum | | 22.64 | 23.92 | 22.34 | 23.51 | 22.74 | 23.93 | 22.72 | 23.87 |

Norra: (1) Aluminum dish for weighing fat.

(2) Average.

(3) 4-5 extractions made.

(4) On same subdivision as Leavitt.

(5) Rogavitz and Goldberg analysed same subdivision.

(6) Stone and Gungy

(7) Ayer and Haydon

Test was also made of a method proposed by W. M. Goldberg, which called for a 10 gram sample, making up to 200 cc. with water, ammonia, and alcohol in the proportions used in the tentative method, and taking a 20 cc. aliquot for the determination. The results obtained, with and without a second addition of alcohol before the second extraction, are given in the last two columns of Table 1. While this method gave fairly good results at the hands of its author, the other analysts found it impossible to work up a 10 gram sample into a homogeneous mixture by following the instructions. Accordingly it was decided to confine the collaborative work to the first two methods.

Each of two samples of whole milk powder was thoroughly mixed, carefully subdivided, and sent to the collaborators with a request to determine fat by the tentative method and the modified tentative method. The methods were supplemented by notes as follows:

1. Mix and weigh out charges from each sample at one time, and (if practicable) make a moisture determination at the same time. To minimize changes in humidity, fat determinations should be completed in one day.
2. Mojonnier flasks are recommended. Report the kind of extraction apparatus used.
3. Use a counterpoise flask in weighing. A few grains of sand in the flask will prevent bumping when the ethers are evaporated.
4. If time permits, repeat the fat determinations, omitting the filtration of the ether extractions through a small quick-acting filter.
5. Note any emulsion formation and advise whether vigorous shaking, as called for, is desirable, or whether the liquids are best mixed by a gentle tilting action.
6. Note the time required to dry the fats to constant weight.
7. Confirm the purity of the fat and report percentage of fat both before and after purifying.
8. Comment on experience with these instructions.

The results on moisture and fat are given in Tables 2 and 3, the fat results being calculated also to a moisture-free basis.

The fat percentages tabulated are those reported as purified when given by the collaborator. Twelve of the collaborators found the correction for the purity of fat nil or negligible. The differences between crude and purified fat reported are given in Table 4.

Notwithstanding the general findings that the fat extracted is pure, the large corrections in some instances make it evident that the analyst should confirm the purity of the fat. Those analysts reporting impurities in the extracted fat found the quantities greater when filters were not used. On the other hand many of the collaborators found no impurities when filters were not used. The presence of impurities in the fat is believed to be due to inadequate settling of the shaken liquids or to going too far in pouring off the ether layer. When three extractions are made it is not necessary to pour off the ether to the last drop.

TABLE 4.
Differences between crude and purified fat.
(Expressed in percentage.)

| COLLABORATOR | SAMPLE NO. 1 | | | | SAMPLE NO. 2 | | | |
|-----------------------------|--------------------------------------|------------------------------|------------------------------|------------------------------|--|------------------------------|--------------------------|------------------------------|
| | Tentative method | Modified method | Tentative without filter | Modified without filter | Tentative method | Modified method | Tentative without filter | Modified without filter |
| Ferris | | | 0.07 | | | | | |
| Rogavitz | | | | 0.07 0.06 | | | 0.21 | |
| Goldberg | | | | 0.07 0.06 | | | 0.10 0.22 | 0.18 |
| Reed | 0.24 | | | 0.04 | 0.14 | 0.01 0.05 | 0.25 | 0.02 |
| Haynes | 0.25 0.33 | 0.17 0.17 | 0.20 0.45 0.12 | 0.22 0.15 | 0.26 0.30 0.50 | 0.30 0.22 0.42 0.43 | 0.15 0.18 0.30 | 0.32 0.52 0.48 |
| Keister | 0.07 0.03 0.20 | 0.24 0.35 | | | 0.03 | 0.12 | | |
| Pruitt | 0.12 0.48 | 0.21 0.09 | | | 0.20 3.42 | 0.18 0.25 | | |
| Ayer | 0.23 0.05 0.05 0.15 0.05 | 0.38 0.23 0.06 0.10 | 0.75 0.88 1.58 0.82 | 4.15 4.35 | 0.08 0.02 0.22 0.05 0.50 2.64 | 0.93 2.15 | 1.70 1.70 | 5.53 2.68 3.88 0.78 |
| Haydon | 0.12 0.06 0.09 | 0.12 0.60 0.06 1.50 | 0.33 0.22 0.29 2.00 | 1.59 0.73 0.44 1.28 | 0.04 | 0.06 0.03 | 0.02 | 0.05 1.61 1.95 |
| Average of above Average | 0.16 0.05 | 0.31 0.09 | 0.84 0.19 | 1.02 0.33 | 0.60 0.17 | 0.37 0.12 | 0.48 0.12 | 1.50 0.44 |

The results in Tables 2 and 3 show conclusively the superiority of the modified method over the present tentative method. This is shown better in Sample No. 1, which was an old and slightly rancid powder. The results by the tentative method on this sample were not only lower, but there was considerable trouble with emulsion formation. Six collaborators reported trouble with emulsions, mostly with the tentative method on Sample No. 1.

The collaborative work also showed that it may be possible to omit the filtering of the ether extracts if the proper technic is employed.

However, the analyst should use filters unless he is certain that he can obtain pure fats without them. It is always necessary to verify the purity of the extracted fats.

All the collaborators except one used Mojonnier flasks for the fat extraction. Fairly vigorous shaking appears necessary to obtain a complete fat extraction. When the modified method is used there is little danger of emulsion formation. The usual time required to dry the fats to constant weight was from 1 to 2 hours. The preparation of the sample for extraction was the cause of considerable comment. There is need of study to determine the best time and temperature of heating. Perlman suggests 3 minutes at 60°; Salinger finds this inadequate for best results, and claims that 25 minutes at 60°-70° is necessary; and Stone and Gnagy heat for 12 minutes on the steam bath.

The transfer of the hydrolized solution from the beaker to the extraction apparatus is a difficult operation because excessive fat separation usually occurs. Perlman and the associate referee's laboratory rinse the beaker with the alcohol and with the ethers used for the extractions. Ferris, Pruitt and Dunn suggest weighing the sample directly into the Mojonnier tube. The Ferris procedure is as follows: Weigh the sample into a small lead boat, which is introduced into the extraction flask. Add water and ammonia and shake quickly; immerse the flask in hot water and shake occasionally until a smooth emulsion is produced. Add alcohol and proceed as in the tentative method.

Dried milk is very hygroscopic, and samples should be weighed out quickly. This is shown by the following results obtained by Stone and Gnagy at Minneapolis on successive days:

| [DATE 1920 | SAMPLE NO. 1 | | SAMPLE NO. 2 | |
|-------------|------------------------|-------|--------------|-------|
| | STONE | GNAGY | STONE | GNAGY |
| | PERCENTAGE OF MOISTURE | | | |
| Sept. 26 | 3.78 | 3.62 | 4.83 | 4.61 |
| " 27 | 3.93 | 3.84 | 4.89 | 4.71 |
| Oct. 1 | 3.94 | 3.85 | 4.95 | 4.71 |
| " 2 | 3.83 | 3.94 | 4.85 | 4.85 |
| " 3 | | 3.90 | | 4.77 |
| " 4 | 4.04 | | 5.02 | |
| " 7 | 4.07 | 3.93 | 5.04 | 4.78 |

CONCLUSIONS.

The present tentative method for fat in dried milk does not give sufficiently accurate or reliable results when used on whole milk powder.

The modification tested gives better results.

The procedure of extraction gives fat in a generally pure condition and is satisfactory.

The tentative method is not sufficiently definite in details of manipulation for the preparation of the sample for extraction, especially as to time and temperature of heating and in transfer of material to the extraction apparatus.

The problem of fat determination in dried milk has three phases: (1) sampling; (2) preparation of sample for extraction; (3) extraction with solvents and recovery of fat. The third phase appears to have been adequately studied. The second is in need of considerable study as indicated in the previous paragraph. When this has been accomplished, attention should be given to the matter of adequate and representative sampling of milk powders, including the handling of larger samples if possible.

The associate referee is highly appreciative of the interest taken in this work by the large number of collaborators. The data and suggestions submitted by them were so extensive that it was difficult to condense them to the confines of this report. Assistance and advice by H. D. Grigsby, U. S. F. D. I. Administration, New York, is also acknowledged.

RECOMMENDATIONS¹.

It is recommended—

(1) That the tentative method for the determination of fat in dried milk² be amended by adding the following: "After the first extraction with ether, add 4 cc. of 95 per cent alcohol to the liquid remaining in the extraction apparatus, mix, then proceed with the second extraction. In the third extraction add, if necessary, sufficient water to raise the level of the aqueous layer to its original volume.

(2) That the same method be amended by inserting the words "Mojonnier flask" before "Röhrig tube".

(3) That further study be made of the details of the tentative method for the determination of fat, including time and temperature of heating with ammonia and avoidance of loss of fat during transfer to the extraction apparatus.

(4) That study be made of the sampling of dried milks.

No report on malted milk was given by the associate referee.

No report on ice cream was given by the associate referee.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 68 (1930).
² *This Journal*, 8, 482 (1925).

REPORT ON MILK PROTEINS.

By H. C. WATERMAN (Office of Experiment Stations, Washington, D. C.),
Associate Referee.

Reports of trials of the associate referee's method¹ for the determination of casein in milk, with filtration modified as suggested by Keister, were secured from two of the collaborators. W. B. White, New York State Department of Agriculture and Markets, reports results obtained by J. L. Perlman, who finds Keister's modification of the filtration satisfactory with regard to clearness of filtrate, but very slow; calls attention to the fact that funnels and beakers should be directed to be covered to prevent evaporation; and recommends filtration with the assistance of diatomaceous earth as follows:

After the precipitated sample had cooled to room temperature it was transferred to a beaker—without washing—and one gram of ignited diatomaceous earth was added. This mixture was thoroughly stirred over the period of 30 minutes at intervals of 5 minutes, the beaker being kept covered with a watch-glass between intervals of stirring. It was then again well mixed and filtered, by means of gentle suction, through a 9 cm. Munktell 00 filter paper on a small Büchner funnel. The liquid was carefully poured on the center of the paper and allowed to moisten it gradually until the suction was equalized. The first 25 cc. of filtrate was returned to the filter.

This filtering procedure required about one minute and the resultant filtrate showed no turbidity. The results with this filtrate were only slightly lower than those secured by using the filtrates obtained in the regular way with Keister's modification. These lower results are probably due to the absence of the small amounts of suspended matter and to the fact that evaporation was minimized by the rapid filtration.

The results are shown in the accompanying table:

TABLE 1.
Results of casein determinations in milk.
(Expressed in grams per 100 cc.)

| TOTAL PROTEIN "A" N × 6.38 | CASEIN— OFFICIAL METHOD | CASEIN BY PROPOSED METHOD | | DIFFERENCE*— AVERAGE |
|-------------------------------|----------------------------|---|-------------------|-------------------------|
| | | Protein in Filtrate "B" | Casein (A - B) | |
| 3 08 | 2.26 | 0.72 | 2 38 | -0 08 |
| 3.12 | 2.30 | 0.72 | 2 38 | |
| | | 0.75 | 2 35 | |
| | | 0.78 | 2 32 | |
| | | SUBSTITUTING DIATOMACEOUS EARTH FILTRATION | | -0.13 |
| | | 0.70 | 2.40 | |
| | | 0.70 | 2.40 | |
| | | 0.67 | 2 43 | |

* Between official and proposed methods.

W. F. Reindollar, Maryland Department of Health, reports that the second filtration in this method proceeds more rapidly when two soft

¹ *This Journal*, 10, 261 (1927).

papers are substituted (as recommended by Keister) for the single hardened filter; that "practically clear" filtrates are obtained with the double filter; and that if a paper of the grade of Whatman No. 1 be carefully fitted to the funnel it is believed that a liquid of such a degree of clarity as to render a second filtration unnecessary can be obtained. Reindollar reports the following results:

| | ORIGINAL METHOD | | KEISTER VARIATION | |
|------------------|-----------------|-----------------|-------------------|-----------------|
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Casein | 2.48 | 2 47 | 2 47 | 2 49 |

RECOMMENDATIONS¹.

In view of the variety of procedures considered desirable by various chemists who have tried this method, the associate referee recommends (1) that the method be modified by substituting for the present specific directions for filtration the words "filter clear, taking care to prevent evaporation during filtration"; (2) that the method as modified be adopted as tentative; and (3) that further collaborative trials of the method be secured if possible during the ensuing year.

No report on qualitative tests was given by the associate referee.

REPORT ON FATS AND OILS.

By G. S. JAMIESON (Bureau of Chemistry and Soils, Washington, D. C.),
General Referee.

During the past year a collaborative study was made of the combined method for the determination of the Reichert-Meissl and Polenske values, and the Kirschner value, which after thorough investigation was adopted as the official method of the American Chemical Society's Committee on Analysis of Commercial Fats and Oils². The method has been published³.

KIRSCHNER VALUE⁴.

The method for the determination of the Kirschner value is stated to depend upon the solubility of silver butyrate in dilute solutions of silver sulfate (the same would also be true of acids lower in the series) and on the insolubility of the silver salts of acids higher than butyric acid in the series. The chief use of the method is to detect the presence of butterfat in admixture with other fats and oils. Although palm kernel and coconut oils give Kirschner values from about 1 to 1.9 (see remarks below), it should be noted that neither of these products contains butyric or

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 69 (1930).

² *Ind. Eng. Chem.*, 18, 1346 (1926).

³ *This Journal*, 13, 43 (1930).

⁴ *Z. Nahr. Genussm.*, 30, 205 (1905).

other acids lower in the series; these results, however, do indicate the slight solubility of the silver salts of caproic, caprylic or capric acids.

It will be observed that the method directs the titration of the 100 cc. Reickert-Meissl distillate with 0.1 *N* barium hydroxide solution. As it is desirable to get information on the use of 0.1 *N* sodium or potassium hydroxide solution for this purpose, it is requested that an experiment be made with two samples, for one of which one of these solutions is used and for the other barium hydroxide. This method has been published¹.

REMARKS.

In the interpretation of Kirschner values, it is suggested that in the absence of coconut or palm kernel oils or their stearines, only values above 0.5 should be considered as giving indications of the presence of butterfat, and the same applies to values above 2.6 in the case of fat mixtures containing coconut or similar oils.

The five samples submitted to collaborators were as follows: (1) Refined cottonseed oil, (2) refined coconut oil, (3) a mixture containing 50 per cent each of the above-mentioned cottonseed and coconut oil, (4) a mixture of equal weights of the above-mentioned cottonseed oil and clarified butterfat, and (5) a mixture of equal quantities of the same sample of butterfat used in 4 and of the same coconut oil as used in 2 and 3.

Richardson also reported duplicate results for the Reichert-Meissl values for each of the five samples in which a standard barium hydroxide solution was used for the titrations. The results agreed closely with those obtained when he used a standard sodium hydroxide solution for the titration (see table).

In submitting Edeler's report attention was called to the directions which state that the water in the condensers used for these determinations should be at 20°C. and that comparatively few laboratories had water at this temperature in the summer time. Edeler used ice water and recirculated it through the condenser by means of an air lift, using the compressed air regularly available in the laboratory. He wrote in part as follows: "Apparatus for this purpose may be constructed of ordinary laboratory glassware in a number of different ways. * * * We have used compressed air to circulate liquids * * *. However, we have not heretofore used it in connection with controlling the temperature of condenser water and hence it seems worth mentioning".

BLANK DETERMINATIONS.

Of those results reported the range for the Reichert-Meissl corrections was from 0.15 to 2.8 cc. of standard alkali solution, and for the Kirschner value determination, from 0.1 to 2.2 cc. The majority reported a blank

¹ *This Journal*, 13, 44 (1930).

Table of results.

| ANALYST | REICHERT-MEISSEL VALUE | | POLENSKE VALUE | | KIRSCHNER VALUE | | | |
|------------------|---------------------------|-------|-------------------|-------|------------------------------------|-------|------------------------------------|-------|
| | | | | | By sodium hydroxide solution | | By barium hydroxide solution | |
| SAMPLE 1 | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) |
| A. Edeler | 0.70 | 0.70 | 0.40 | 0.40 | 0.45 | 0.50 | 0.40 | 0.50 |
| J. T. Keister | 0.12 | 0.00 | 0.60 | 0.55 | 0.49 | | 0.35 | |
| S. I. Gertler | 0.14 | 0.18 | 0.35 | 0.45 | 0.48 | | 0.38 | |
| W. D. Richardson | 0.44 | 0.41 | 0.22 | 0.22 | 0.32 | 0.30 | 0.34 | 0.34 |
| M. L. Sheeley | 0.44 | 0.44 | 0.40 | 0.40 | | | 0.12 | 0.12 |
| F. Fenger | 0.41 | 0.34 | 0.22 | 0.20 | 0.17 | 0.17 | 0.15 | 0.15 |
| SAMPLE 2 | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) |
| A. Edeler | 6.70 | 6.30 | 15.10 | 16.00 | 2.00 | 2.10 | 1.80 | 1.75 |
| J. T. Keister | 6.02 | 5.93 | 15.26 | 15.27 | 2.25 | | 3.07 | |
| S. I. Gertler | 6.88 | 6.93 | 12.30 | 12.85 | 3.01 | | 2.61 | |
| W. D. Richardson | 6.79 | 6.79 | 16.22 | 16.12 | 2.42 | 2.42 | 2.41 | 2.41 |
| F. Fenger | 6.31 | 6.35 | 13.80 | 13.70 | 2.38 | 2.43 | 2.54 | 2.54 |
| SAMPLE 3 | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) |
| A. Edeler | 5.20 | 5.00 | 6.50 | 6.50 | 1.00 | 0.90 | 1.20 | 1.05 |
| J. T. Keister | 4.48 | 4.35 | 6.49 | 6.07 | 0.82 | | 0.82 | |
| S. I. Gertler | 4.79 | 4.57 | 6.45 | 6.70 | 3.22 | | 2.56 | |
| W. D. Richardson | 5.28 | 5.25 | 6.42 | 6.32 | 1.34 | 1.42 | 1.18 | 1.24 |
| M. L. Sheeley | 3.60 | 3.50 | 3.80 | 3.70 | | | 0.36 | 0.49 |
| F. Fenger | 4.75 | 4.82 | 5.82 | 6.06 | 1.32 | 1.44 | 1.43 | 1.50 |
| SAMPLE 4 | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) |
| A. Edeler | 14.20 | 13.80 | 1.25 | 1.20 | 11.60 | 11.50 | 11.60 | 11.20 |
| J. T. Keister | 14.00 | 13.89 | 0.95 | 0.85 | 13.80 | | 13.62 | |
| S. I. Gertler | 13.70 | 13.70 | 1.05 | 1.05 | 12.50 | | 12.60 | |
| W. D. Richardson | 14.40 | 14.50 | 0.72 | 0.80 | 12.40 | 12.30 | 12.65 | 12.67 |
| M. L. Sheeley | 13.86 | 14.30 | 0.90 | 1.00 | | | 13.90 | 13.80 |
| F. Fenger | 14.05 | 14.35 | 0.56 | 0.66 | 12.83 | 12.66 | 12.77 | 12.77 |
| SAMPLE 5 | (1) | (2) | (1) | (2) | (1) | (2) | (1) | (2) |
| A. Edeler | 18.20 | 17.80 | 7.15 | 7.40 | 12.50 | 12.40 | 12.00 | 12.00 |
| J. T. Keister | 17.00 | 17.45 | 7.90 | 7.80 | 12.00 | | 12.30 | |
| S. I. Gertler | 15.80 | 15.95 | 7.25 | 7.20 | 15.50 | | 15.30 | |
| W. D. Richardson | 18.00 | 18.00 | 8.10 | 8.20 | 13.10 | 13.00 | 12.90 | 12.90 |
| F. Fenger | 17.77 | 17.85 | 6.60 | 6.50 | 12.67 | 12.84 | 12.27 | 12.14 |

amounting to 0.6 cc. or less in the case of the Reichert-Meissl, and 0.1 cc. for the Kirschner determination.

As has long been known, satisfactory results for both the Reichert-Meissl and the Polenske determinations can only be obtained by those who actually follow in every detail the "set up" of the apparatus and the technic of these procedures as described, and that to master these methods requires much experience. In view of these facts the results, with some very obvious exceptions, show fair agreement.

As little or nothing would be gained by continuing the collaborative study of these procedures, which previous study by many others, including the referee, has shown give good results, they will be recommended for adoption, with the provision that in the case of the Kirchner value the titration of the fatty acids may be made by either barium hydroxide, sodium, or potassium hydroxide solutions.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the "cold test" be made official (first action).
- (2) That the lead-salt-ether method be made official (final action).
- (3) That the combined Reichert-Meissl and Polenske Method be made official and substituted for the present separate methods under "soluble" and "insoluble volatile acids" in *Methods of Analysis, A. O. A. C.*, with the exception that the illustration of apparatus on p. 292 be retained (first action).
- (4) That the Kirschner method, using standard solutions of sodium, potassium, or barium hydroxide for the titration, as described in this report, be made official (first action).
- (5) That methods for the determination of moisture in fats and oils be studied, with particular reference to the rapid hot-plate procedure.
- (6) That methods for the determination of the hexabromide number (ether insoluble) of drying oils be studied.

REPORT ON BAKING POWDERS AND BAKING CHEMICALS.

By G. L. BIDWELL (Food, Drug and Insecticide Administration,
Washington, D. C.), *Referee*.

Raymond Hertwig called attention to an apparent error in the Chittick method of determining carbon dioxide in baking powders. M. R. Coe of the Food Control Laboratory studied the question and confirmed Hertwig's results. In discussing this matter with others the statement was made that there was no error in the Chittick method since it checked the official Knorr method. Being sure that the error did exist, the referee investigated the Knorr method and found that it involved an error of equal but opposite magnitude to that shown by the Chittick method. Since that time there has been received in the Department of Agriculture a manuscript from one of the Experiment Stations calling attention to this same error.

The errors in methods of this kind seem to be of such importance that the work of the referee was expended on this subject, and it is recommended that further work be done with a view to changing both the Knorr and Chittick methods².

¹ For report of Subcommittee A and action of the association, see *This Journal*, 13, 69 (1930).

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 70 (1930).

CONTRIBUTED PAPERS.

THE USE OF LEAD ACETATE IN THE DETERMINATION OF THE ACIDITY OF FRUIT PRODUCTS.

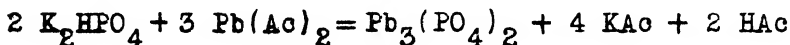
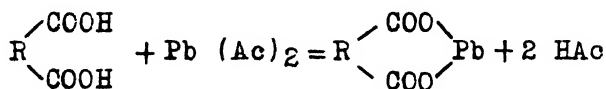
By B. G. HARTMANN and F. HILLIG (Food Control Laboratory¹, Food,
Drug and Insecticide Administration, U. S. Department of
Agriculture, Washington, D. C.).

The acidity of a solution is determined by titration with standard alkali, and several indicators are recommended for determining the point of neutrality. Phenolphthalein is generally used if the material under examination is colorless, and litmus tincture (spot method) and phenolphthalein powder are employed to advantage in the case of highly colored solutions². If a large quantity of a coal tar dye is present, the removal of the dye with wool, with subsequent titration with phenolphthalein as the indicator, has been recommended by Badger and Sale³.

It is well known, however, that in the titration of acid phosphates, normal constituents of fruits, the third hydrogen is not indicated with phenolphthalein. According to Clark⁴ the third hydrogen atom of phosphoric acid is indicated in electrometric titrations at about pH 12, whereas phenolphthalein changes at a pH ranging from 8.3 to 10.0. Obviously then, in a solution that contains a large quantity of added phosphoric acid (artificial jams and jellies), the total free acidity may materially exceed the titratable acid obtained with phenolphthalein.

In this paper the authors show that phenolphthalein is a very satisfactory indicator for organic acids and describe a method that accurately determines free acidity inclusive of the acidity due to phosphates.

When lead acetate is added to a solution of a fruit acid the neutral lead salt of the acid is formed and the equivalent quantity of acetic acid is liberated.



The liberated acetic acid is a true measure of the acidity, and with the precipitation of the lead salts a substantial portion of the coloring

¹ The late R. W. Balcom, Chemist in Charge.

² *Methods of Analysis*, A. O. A. C., 1925, 365.

³ *J. Assoc. Official Agr. Chem.*, 9, 343 (1926).

⁴ *The Determination of Hydrogen Ions*, 3rd ed., pp. 28 and 82.

matter of the fruit is removed. Accurate titrations on a de-leaded portion of the filtrate are then made possible.

Based upon these considerations, the following method for the determination of the acidity of fruit products was formulated. The addition of the small quantity of nitric acid is necessary to stabilize the reaction.

PROPOSED METHOD FOR THE DETERMINATION OF THE ACIDITY OF FRUIT PRODUCTS.

PREPARATION OF SOLUTION.

If the material to be analyzed contains insoluble solids, prepare a sample solution as directed in *Methods of Analysis*, A. O. A. C., 1925, p. 209. (It is, of course, unnecessary to prepare a sample solution of fruit juices.) For the determination of the acidity of a fermented beverage, remove the alcohol, because the coloring matter of the fruit is soluble in alcohol. Bring to incipient boiling such products as carbonated soft drinks to remove carbon dioxide.

REAGENTS.

Nitric acid solution.—Dilute 15 cc. of concentrated nitric acid to 1 liter with boiled distilled water.

Lead acetate solution.—Dissolve 100 grams of normal lead acetate in 300 cc. of boiled distilled water containing 10 cc. of glacial acetic acid. Boil for 10 minutes, cool, make to 1 liter with boiled distilled water, and filter.

DETERMINATION.

Transfer 200 cc. of the sample solution, or 25 cc. of the fruit juice, to a 250 cc. volumetric flask and adjust the volume, if necessary, to about 200 cc. with boiled distilled water. Add 20 cc. of the nitric acid solution and shake. Then add 20 cc. of the lead acetate solution, shake, make to mark, and filter. Add dry potassium oxalate to the filtrate to remove lead, being careful not to add a large excess, and re-filter. Titrate 100 cc. of the filtrate with 0.1 *N* sodium hydroxide, using phenolphthalein as the indicator. Determine the acidity of the reagents used by running a blank in the same manner as in the procedure.

Data obtained on the organic acids that are naturally contained in fruit products, or that may have been added in the process of manufacture, are presented in Table 1. The data on the neutral salts are included to show that these salts are neutral to lead acetate titration. The salts of the respective acids were obtained by the addition of the required quantity of potassium hydroxide.

As stated previously, the total available acidity of phosphoric acid is not indicated by direct titration. In Table 2, however, data are presented to show that the lead acetate method does really determine the available acidity of phosphoric acid and its acid salts. The solutions were prepared from C. P. ortho phosphoric acid. The percentage of phosphoric acid in the solution was determined by specific gravity and verified by the volumetric ammonium molybdate method. The salts were prepared by adding potassium hydroxide to the phosphoric acid solution in the required quantities to form the various salts.

TABLE 1.

Comparison of results obtained by using the lead acetate and direct titration methods on organic acids and their salts.

| SOLUTION | MATERIAL IN SOLUTION | TITRATABLE ACID | |
|-----------------------------------|----------------------------|---------------------|------------------------|
| | | Direct Titration | Lead Acetate Method |
| | <i>gram</i> | <i>gram</i> | <i>gram</i> |
| Tartaric acid..... | 0.216 | 0.216 | 0.214 |
| Potassium hydrogen tartrate | 0.271 | 0.108 | 0.106 |
| Potassium tartrate..... | 0.325 | 0.000 | 0.000 |
| Malic acid..... | 0.198 | 0.198 | 0.197 |
| Potassium hydrogen malate..... | 0.255 | 0.099 | 0.097 |
| Potassium malate..... | 0.310 | 0.000 | -0.002 |
| Citric acid..... | 0.189 | 0.189 | 0.190 |
| Potassium dihydrogen citrate..... | 0.226 | 0.126 | 0.127 |
| Potassium hydrogen citrate..... | 0.264 | 0.063 | 0.065 |
| Potassium citrate..... | 0.301 | 0.000 | -0.002 |
| Salicylic acid..... | 0.198 | 0.198 | 0.192 |
| Potassium salicylate..... | 0.252 | 0.000 | -0.004 |
| Lactic acid..... | 0.214 | 0.214 | 0.210 |
| Oxalic acid..... | 0.144 | 0.144 | 0.141 |
| Benzoic acid..... | 0.198 | 0.198 | 0.198 |
| Succinic acid..... | 0.199 | 0.199 | 0.198 |

As was to be expected, the direct titration does not indicate the available acidity of phosphoric acid, whereas the lead acetate method permits of accurate determination.

Since one of the objections to the methods for determining acidity by direct titration is the interference of coloring matter, experiments were conducted on acid solutions of known acidity to which permitted coal tar dyes had been added. No difficulty was experienced in securing a sharp end point when the lead acetate method was applied.

Results obtained on commercial jams, jellies and fruit juices are presented in Table 3.

TABLE 2.

Comparison of the results obtained by using the lead acetate and direct titration methods on phosphoric acid and its salts.

| SOLUTION | MATERIAL IN SOLUTION | TITRATABLE ACID | | |
|--------------------------------------|----------------------------|---------------------|------------------------|-------------|
| | | Direct Titration | Lead Acetate Method | Theoretical |
| | <i>gram</i> | <i>gram</i> | <i>gram</i> | <i>gram</i> |
| Phosphoric acid..... | 0.138 | 0.088 | 0.137 | 0.138 |
| Potassium dihydrogen phosphate | 0.191 | 0.046 | 0.089 | 0.092 |
| Potassium hydrogen phosphate..... | 0.245 | 0.003 | 0.044 | 0.046 |
| Potassium phosphate..... | 0.289 | 0.000 | +0.003 | 0.000 |

TABLE 3.

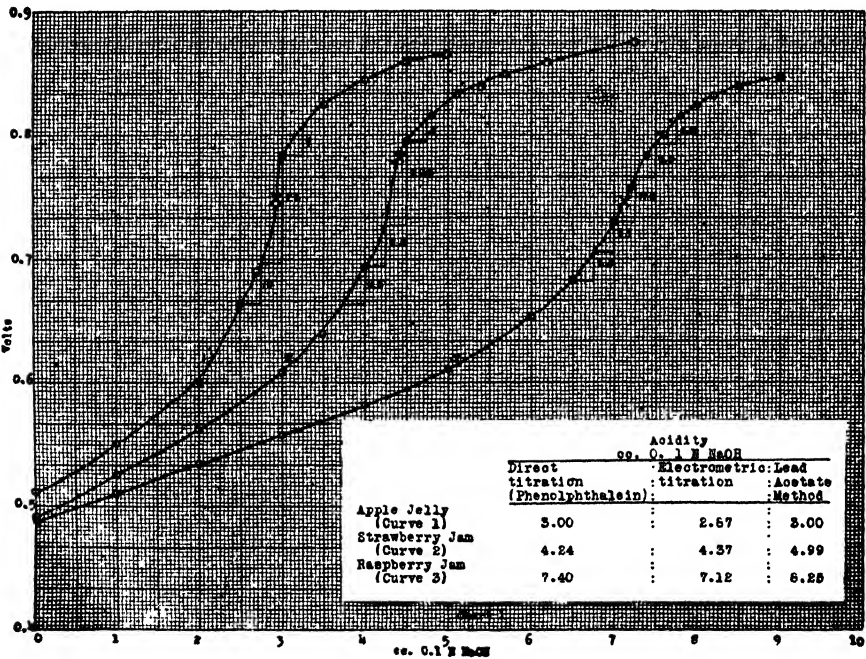
Comparison of results obtained by using the lead acetate and direct titration methods on commercial fruit products.

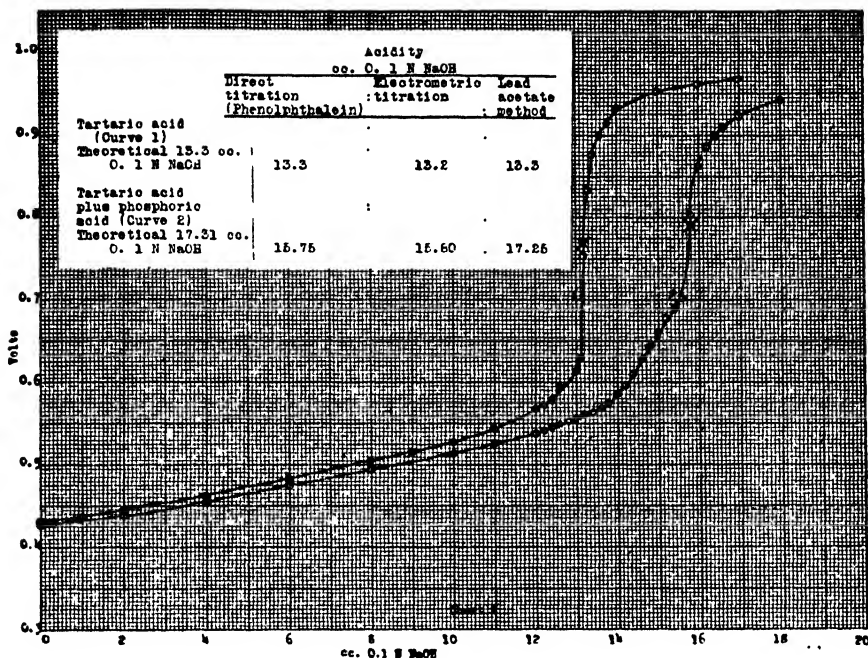
| KIND OF PRODUCT | ACIDITY | |
|-----------------------|--|---|
| | Direct Titration cc. 0.1 N HCl per 100 grams | Lead Acetate Method cc. 0.1 N HCl per 100 grams |
| Blackberry jam..... | 61 | 70.9 |
| Raspberry jam..... | 99 | 110.0 |
| Strawberry jam..... | 57 | 65.9 |
| Apple jelly..... | 40 | 40.0 |
| Imitation jelly*..... | 64 | 91.7 |
| Grape juice..... | 153 | 162.4 |
| Grapefruit juice..... | 218.6 | 220.6 |

* Cane sugar, corn sirup, apple pectin, color and phosphoric acid added.

The data in Table 3 show that the lead acetate method yields higher acidities than does the direct titration method. There is no doubt that this difference is due mainly to the presence of phosphoric acid or to its acid salts. A sharp end point was obtained in all cases when the lead acetate method was used.

The method was also tried on the esters, methyl anthranilate and ethyl acetate. Both direct titration and titration after treatment with lead acetate indicated that the esters were practically neutral.

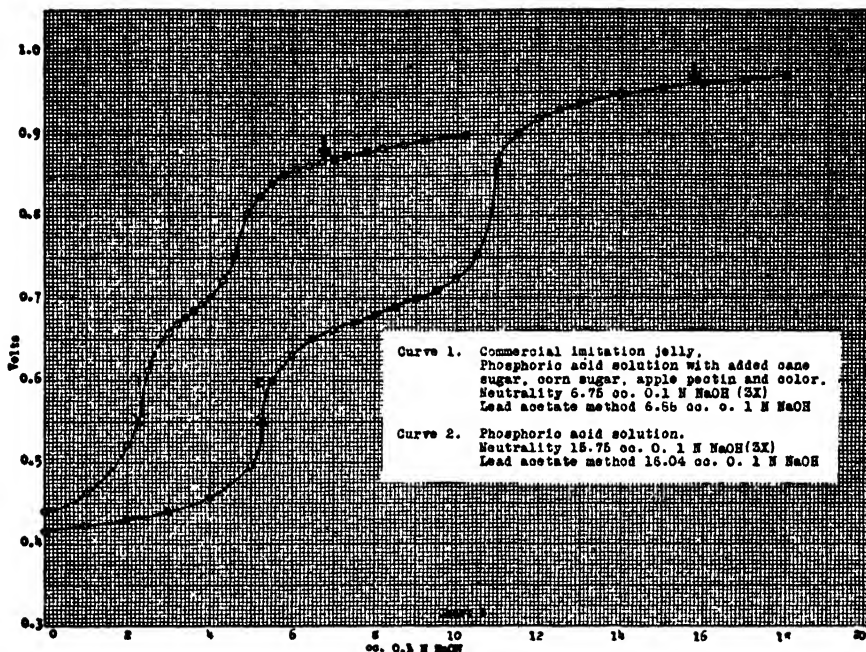




In the hope that the hydrogen electrode might serve the purpose of verifying the results obtained by the lead acetate method, determinations were made on some of the more common fruit products by this means. The solutions necessary for a comparison of the three methods under consideration were prepared according to *Methods of Analysis*, A. O. A. C., p. 209, and 50 cc. was used for the titrations. For the electrometric titration the Bunker type electrode vessel was used, and a Leeds and Northrup Type K potentiometer was employed as the measuring instrument.

Chart I represents electrometric titration curves on apple jelly, strawberry jam and raspberry jam. The supplementary data show that the hydrogen electrode titration and direct titration agree very closely. However, it is believed that the results obtained by the lead acetate method, which are materially higher, are more nearly correct, as shown conclusively in Chart 2, which is self-explanatory. Chart 3 shows that phosphoric acid solution or compounds consisting primarily of phosphoric acid may be accurately titrated electrometrically.

In explanation of the higher values obtained by the lead acetate method over those obtained by the electrometric method, it should be remembered that the latter measures the ionization of the acid present, and consequently the difference between the results obtained represents the unionized acid. From a theoretical viewpoint, it would seem that



the lead acetate method gives the available acidity. When lead acetate reacts with an acid, the available hydrogens are replaced by lead, the lead salt of the acid is formed, and the equivalent acetic acid liberated is titrated in the proposed method by using phenolphthalein. According to Wendt¹, phenolphthalein is an excellent indicator for acetic acid.

No mention has been made of one of the characteristic constituents of fruit products, namely, tannin, and there is no concise information in the literature regarding it. As a matter of interest, experiments were made with digallic acid, the ordinary U. S. P. tannin of the market. Determinations on 200 mg. of this acid in aqueous solution required 4.5 cc. and 15.25 cc. of 0.1 N sodium hydroxide, respectively, when titrated directly and after treatment with lead acetate. It was observed that hydrolysis occurred in the direct titration of the acid and that the titer gradually approached that indicated by the lead acetate method. It would seem, therefore, that lead acetate breaks up the lactone formation of digallic acid, thereby liberating the carboxyl group. Whether the tannin occurring in fruits is the lactone or the acid is not known, and therefore it is not possible to state how lead acetate affects the acidity due to tannin compounds.

SUMMARY.

A new chemical method is described for the accurate and rapid determination of the acidity of fruit products. The natural coloring matter

¹ Leach. Food Inspection and Analysis, 4th ed., p. 1031.

of a fruit product, or added color in moderate quantities, does not interfere with the end point determined with phenolphthalein. The method indicates the replacement of the third hydrogen atom of phosphoric acid. The direct titration and the electrometric titration do not give the available acidity of a fruit product, owing to the incomplete ionization of the fruit acids.

The authors wish to express their appreciation to L. E. Dawson, of the Bureau of Chemistry and Soils, Department of Agriculture, Washington, D. C., for his helpful suggestions in preparing the material covering the electrometric titrations.

THE DETERMINATION OF CAFFEINE IN DECAFFEINATED COFFEE¹.

By WINSTON F. ALLEN (Michigan State College, East Lansing, Mich.).

INTRODUCTION.

The determination of caffeine in coffee has held the attention of the Association of Official Agricultural Chemists since 1908². The Fuller, Gorter, Hilger and Fricke, Stahlschmidt, and other methods were investigated by referees of the association, and all were discarded except the Stahlschmidt method, which was adopted provisionally in 1915. The Fendler-Stüber method replaced the Stahlschmidt method in 1921 as a tentative method, and in 1923 the Power-Chesnut method was added as the official method for the determination of caffeine in both tea and coffee. The Fendler-Stüber and the Power-Chesnut methods are the most satisfactory of any thus far studied. In his report as Referee on Coffee in 1920³, H. A. Lepper recommends the Power-Chesnut method because of its scientific accuracy and wide adaptability, but states that the Fendler-Stüber method should be retained as a tentative method because it is especially applicable when quick results are desired and when no special apparatus is available. While both methods are said to be adaptable to the determination of caffeine in decaffeinated coffee, no mention is made in the procedures of any precautions to be observed when such an analysis is made.

These two methods calculate the percentage of caffeine on the weight of the final residue that is left after evaporating the chloroform and drying in an oven at 100°C. for 30 minutes. The analyst may or may not ascertain the purity of this caffeine residue by determining its nitrogen content and multiplying by the factor 3.464. The percentage of caffeine may be based upon the weight of the residue and be considered correct. The error here, due to the oily and waxy impurities present with the

¹ Contribution from the Kedsie Chemical Laboratory, Michigan State College.

² *This Journal*, 1, 203 (1915-16).

³ *Ibid.*, 5, 273 (1921-22).

caffeine, is perhaps negligible with ordinary coffee, which often gives a 95 per cent pure caffeine residue, while in the case of decaffeinated coffee the final caffeine residue is usually only from 20 to 40 per cent pure caffeine. Basing the percentage of caffeine in this case upon the weight of the residue is entirely erroneous.

It was the object of this project to study modifications of the methods discussed in order to make them more fully applicable to the analysis of decaffeinated coffee.

Two commercial brands of decaffeinated coffee were used as samples. In the tables they are designated as Product I and Product II.

METHODS.

The official Power-Chesnut and the tentative Fendler-Stüber methods¹ were used in this work. The procedure for the Power-Chesnut method was followed exactly as given, but the Fendler-Stüber method was modified as follows:

1. All of the first chloroform extract was used instead of an aliquot. By holding the stopper partially in place when pouring the chloroform into the funnel and using a cotton plug instead of a large filter paper, practically all the residue was retained in the bottle. This procedure eliminated two weighings and the cooling in ice, but it necessitated the shaking of the residue with three small portions of chloroform, which was done quite rapidly. (The average of 10 samples was 0.0158 per cent caffeine when an aliquot was taken, and 0.0167 per cent when the entire extract was taken.)

2. An ordinary small quantitative filter paper was used instead of a Gooch crucible to filter the aqueous solution after oxidation with potassium permanganate. This was more convenient when a number of samples were run at the same time.

3. After each shaking the final chloroform extract was filtered through a small filter paper as it was being run from the separatory funnel. This prevented filmy particles and water from getting into the chloroform extract and slightly increased the purity of the caffeine residue.

METHODS OF DETERMINING THE PURITY OF THE CAFFEINE RESIDUE.

A.—Nitrogen determination.

The three following methods were investigated:

1. A micro-Kjeldahl method similar to Pregl's procedure² was employed in obtaining the results shown in Table 1. This method is essentially a refinement of the official Kjeldahl-Gunning-Arnold procedure, the exception being the use of steam distillation. However, the Pregl procedure was modified by using a special apparatus (of Pyrex

¹ *Methods of Analysis*, A. O. A. C., 1925, 334.

² *Quantitative Organic Microanalysis*, 2nd ed., 1924, p. 94.

glass), which was found to be very convenient and time saving. The digestion of the caffeine residue was made in 200 cc. Florence flasks after evaporating the chloroform. These flasks were connected directly with the apparatus for digestion. Thus it may be noted that two transfers of the material were eliminated. The design was such that the distillation could be accomplished rapidly and with no danger of sodium hydroxide being carried over mechanically, as seemed likely when the Parnas and Wagner modification of Pregl's apparatus was used in some of the earlier analyses.

2. The ordinary macro-Kjeldahl apparatus was found to give satisfactory results, providing the same digestion mixture and $N/100$ solutions were used as with the micro procedure. It was found to be very important to steam out the apparatus thoroughly immediately before using. This was done until the blank determinations did not vary more than 0.30 cc. of $N/100$ acid used. When the steaming of the macro apparatus was omitted, blank determinations varied greatly, and in some cases they neutralized as much acid as would be required in the determination of the caffeine residue obtained from the average sample of decaffeinated coffee.

3. Direct Nesslerization by the Koch-McMeekin method¹ was contemplated as a possible method of determining nitrogen in caffeine. The average of six samples analyzed by this method gave 0.0158 per cent caffeine by direct Nesslerization, and 0.0173 per cent by the micro-Kjeldahl procedure. There was considerable variation with some of the samples; hence direct Nesslerization was discontinued in favor of the micro-Kjeldahl method.

B.—Sublimation.

A two-piece sublimation tube made from a 25 x 200 mm. Pyrex test tube, as shown in the accompanying drawing, was found to work satisfactorily for the quantitative sublimation of caffeine. The following procedure, which insures complete sublimation of comparatively pure caffeine, was developed:

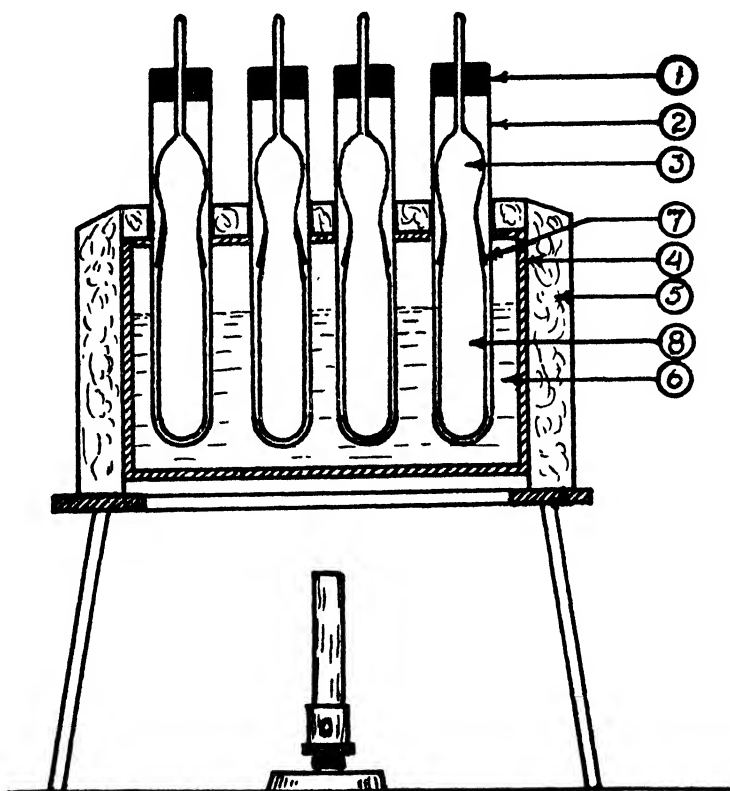
1. The residue of caffeine was washed into the lower half with several small portions of chloroform. The solvent was carefully evaporated off on the steam bath, the tube being kept in an almost horizontal position in order to leave the residue in a very thin layer. The last traces of chloroform were removed by a gentle current of air.

2. The two parts were put together and placed in a 30 x 200 mm. test tube. A rather loose fitting cork was put into the end of the outside tube. The assembled apparatus was so placed in a paraffin bath that the ground-glass joint was about 2 cm. below the surface of the

¹ *J. Am. Chem. Soc.*, 46, 2066 (1924).

liquid. Thus sublimation took place in an air bath contained in a paraffin bath. The temperature was maintained at 180°–190°C. for at least 10 hours.

3. After sublimation was complete the upper tube was immediately removed, cooled, and weighed. The crystals were washed out of the tube with several small portions of chloroform, and the empty tube was dried, cooled, and reweighed. If the nitrogen determination was to be made, the crystals were washed directly into the 200 cc. flask used for digestion.



SUBLIMATION APPARATUS FOR THE QUANTITATIVE DETERMINATION OF CAFFEINE.

1. Loose fitting cork stopper.
2. 80 x 200 mm. test tube.
3. Upper half of sublimation tube containing the sublimed caffeine crystals. Made from a 25 x 200 mm. Pyrex test tube.
4. Cast iron tank.
5. Asbestos jacket.
6. Paraffine bath kept at 180°C.
7. Ground glass joint.
8. Lower half, containing the residue from the crude caffeine, also made from a 25 x 200 mm. Pyrex test tube.

While some literature¹ regarding the quantitative sublimation of caffeine specifies a temperature of 220°–240°C. for about 2–3 hours, a

¹ See Additional References, p. 272.

TABLE 1.

Comparative results, expressed in percentage, of caffeine analyses of decaffeinated coffee when based upon the weight, nitrogen determination, and sublimation of the caffeine residue.

| SAMPLE NO. | FENDLER-STÜBER METHOD | | | | POWER-CHEENUT METHOD | | | |
|------------|-----------------------|---------------------------|----------------|---------------------------|----------------------|---------------------------|----------------|---------------------------|
| | By weight of residue | By nitrogen determination | By sublimation | | By weight of residue | By nitrogen determination | By sublimation | |
| | | | By weight | By nitrogen determination | | | By weight | By nitrogen determination |
| Product I | | | | | | | | |
| 17 | 0.034 | 0.0148 | 0.014 | 0.0102 | 0.062 | 0.0149 | 0.012 | 0.0100 |
| 18 | 0.030 | 0.0125 | 0.012 | 0.0097 | 0.058 | 0.0164 | 0.010 | 0.0072 |
| 19 | 0.062 | 0.0143 | 0.014 | 0.0133 | 0.065 | 0.0204 | 0.015 | 0.0097 |
| 21 | 0.034 | 0.0135 | 0.012 | 0.0131 | 0.059 | 0.0203 | 0.022 | 0.0114 |
| 22 | 0.024 | 0.0103 | 0.010 | 0.0080 | 0.058 | 0.0176 | 0.012 | 0.0063 |
| 23 | 0.053 | 0.0259 | 0.030 | 0.0253 | 0.075 | 0.0318 | 0.024 | 0.0222 |
| 30 | 0.046 | 0.0157 | 0.018 | 0.0113 | 0.048 | 0.0163 | 0.030 | 0.0096 |
| 31 | 0.048 | 0.0141 | 0.007 | 0.0104 | 0.045 | 0.0158 | 0.015 | 0.0086 |
| 32 | 0.058 | 0.0114 | 0.011 | 0.0063 | 0.039 | 0.0128 | 0.021 | 0.0052 |
| 33 | 0.045 | 0.0143 | 0.014 | 0.0102 | 0.048 | 0.0144 | 0.015 | 0.0074 |
| 34 | 0.054 | 0.0150 | 0.020 | 0.0126 | 0.058 | 0.0179 | 0.016 | 0.0125 |
| 35 | 0.044 | 0.0123 | 0.012 | 0.0096 | 0.059 | 0.0145 | 0.020 | 0.0104 |
| 36 | 0.040 | 0.0129 | 0.015 | 0.0093 | 0.043 | 0.0136 | 0.014 | 0.0095 |
| Product II | | | | | | | | |
| 2 | 0.075 | 0.0424 | 0.034 | 0.0369 | 0.060 | 0.0363 | 0.037 | 0.0298 |
| 3 | 0.084 | 0.0461 | 0.039 | 0.0401 | 0.077 | 0.0420 | 0.049 | 0.0310 |
| Average | 0.0487 | 0.0183 | 0.0174 | 0.0151 | 0.0569 | 0.0203 | 0.0208 | 0.0127 |

much lower temperature was found necessary to prevent brown oily material from subliming with the caffeine. Sublimation was proved to be complete by washing the caffeine out of the upper half of the tube, reassembling, and running the temperature up to about 240°C. for 2 hours. Not a trace of sublimed caffeine could be seen. Further evidence of the complete sublimation of the alkaloid, at the temperature of 180°–190°C. for 10 hours, was obtained by subliming 2 mg. samples of pure caffeine. The average of six trials gave a recovery of 2.20 mg. by weight and 1.97 mg. by nitrogen determination. There was very little variation among these samples.

TABLE 2.

Comparative accuracy of the Macro-Kjeldahl and the Micro-Kjeldahl methods of nitrogen determination.

(The digestion mixture, in both methods, consisted of 2 cc. of concentrated H_2SO_4 , 2 grams of K_2SO_4 , and 0.02 gram of $CuSO_4$. Approximately 13 cc. of concentrated $NaOH$ was added before distillation.)

A. Blank determinations (cc.'s of $N/100 H_2SO_4$ neutralized).

| MACRO-KJELDAHL | | | | MICRO-KJELDAHL |
|--------------------|--------------------------------|-----------------------------|---|---|
| Not steamed at all | Steamed before running blanks | | | Steamed at least 15 minutes just before using |
| | ½ hour 3 hours before using | 1 hour just before using | 4 hours intermittently before using | |
| 2.70 | 1.23 | 0.55 | 0.43 | 0.23 |
| 1.71 | 0.63 | 0.59 | 0.50 | 0.16 |
| 0.73 | 0.88 | 0.86 | 0.48 | 0.20 |
| 2.98 | 1.31 | 0.74 | 0.58 | 0.19 |
| 5.00 | 0.75 | 0.84 | 0.51 | 0.21 |
| 1.09 | 0.66 | 0.62 | 0.46 | 0.19 |
| 1.95 | 0.55 | 0.68 | 0.35 | 0.20 |
| Avg. 2.31 | 0.86 | 0.70 | 0.47 | 0.20 |

B. Analysis of samples (percentage of caffeine).

| SAMPLE AND NUMBER | MACRO-KJELDAHL | MICRO-KJELDAHL |
|--------------------|----------------|----------------|
| Product I—16 | 0.0126 | 0.0147 |
| “ 17 | 0.0134 | 0.0148 |
| “ 18 | 0.0113 | 0.0125 |
| “ 23 | 0.0273 | 0.0259 |
| “ 24 | 0.0165 | 0.0153 |
| “ 25 | 0.0136 | 0.0164 |
| Average (per cent) | 0.0158 | 0.0166 |
| Pure caffeine | | |
| gram | | |
| 0.00202 | 0.00202 | 0.00202 |
| “ | 0.00205 | 0.00203 |
| “ | 0.00211 | 0.00201 |
| “ | 0.00199 | 0.00203 |
| “ | 0.00191 | 0.00202 |
| “ | 0.00209 | 0.00202 |
| Average (gram) | 0.00203 | 0.00202 |

The results obtained by sublimation were not quite so accurate as those obtained by the nitrogen determination; the combination of the two methods gave the most accurate results. By running the nitrogen determination on the residue left in the lower tube after sublimation, the quantity of non-caffeine nitrogen was determined. The analysis of 15 samples showed an average of 19.3 per cent non-caffeine nitrogen by the Fendler-Stüber method and 34.2 per cent by the Power-Chesnut method.

SUMMARY.

The Association of Official Agricultural Chemists has a tentative and an official method for the determination of caffeine in coffee, but at the present time there is no official method for this determination in decaffeinated coffee, although the increasing use of this product makes such a method desirable.

When the two recognized methods applicable to coffee are used for the analysis of decaffeinated coffee, a considerable error is introduced if the percentage of caffeine is based upon the weight of the final residue. The A. O. A. C. methods suggest that the purity of the residue may be tested by running the nitrogen determination, but this step is not required.

In a series of analyses of decaffeinated coffee, a great difference in caffeine content was found between the results based upon the weight of the residue and those based upon the nitrogen determination. Thus, 15 samples run by the Fendler-Stüber method gave an average of 0.0487 per cent caffeine by weight and 0.0183 per cent by nitrogen determination; when run by the Power-Chesnut method, the same sample gave 0.0569 per cent caffeine by weight, and 0.0203 per cent by nitrogen determination.

In order to confirm the accuracy of the nitrogen determination, caffeine residues from the samples were quantitatively sublimed to separate the pure caffeine. The weight of caffeine sublimed checked very closely with the nitrogen determination of the same samples. The 15 samples of decaffeinated coffee, when run by the Fendler-Stüber method, averaged 0.0174 per cent caffeine by weight of sublimed caffeine, and 0.0151 per cent by the nitrogen determination after sublimation. When run by the Power-Chesnut method the same samples averaged 0.0208 per cent caffeine by weight, and 0.0127 per cent by nitrogen determination after sublimation.

It can be readily seen that an accurate determination of caffeine in decaffeinated coffee can be effected by a slight modification of the present methods, wherein the caffeine content is computed from a careful nitrogen determination on the final caffeine residue rather than from its weight.

The quantity of nitrogen in the residues obtained from decaffeinated coffee is so small (averaging about 0.5 mg. of nitrogen) that the ordinary Kjeldahl procedure is not applicable unless certain precautions are observed.

In some analyses the more accurate micro-Kjeldahl procedure of Pregl was employed. Investigation has shown, however, that the ordinary Kjeldahl apparatus may be employed providing more dilute standard solutions are used (at least $N/70$) and the distillation apparatus is very thoroughly steamed out immediately before using. The digestion mixture should also be correspondingly reduced.

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1. J. Burmann, *Ann. Fals.*, 4, 99-101 (1911); *Bull. Soc. Chem.*, 7, 239 (1910).
2. E. Phillippe, *Mitt. Lebensm. Hyg.*, 6, 177-91, 233-47 (1915).

THE QUANTITATIVE DETERMINATION OF EGG IN ICE CREAM¹.

By NORMAN C. SMITH, *Assistant Chemist* (California State Department of Agriculture).

PART I.

INTRODUCTION.

In order to enforce that section of the Dairy Law of California² defining French ice cream or egg ice cream and similar frozen products, the California Department of Agriculture, Division of Chemistry, was called upon to provide a method which would be adequate for a quantitative determination of egg in ice cream.

As a result of a comprehensive search of the literature it was found that since 1890 food chemists in Germany and in the United States Bureau of Chemistry had evolved methods for determining eggs in certain foods, including baked goods and noodles, but that nothing had been done toward developing a method for estimating egg yolk in ice cream.

¹ Presented by the author, June 12, 1929, before the Annual Conference of Dairy and Milk Inspectors at Sacramento, Calif.

² Agriculture Statutes State of California; Dairy Laws corrected to Sept. 1, 1929.

Considerable work also has been reported on the composition of eggs and egg products.

Bein¹ and Winchelhaus² were the first to determine egg yolk in food products quantitatively by ascertaining the P_2O_5 in the alcohol-ether extract. Juckenack and Pasternack³ undertook to solve the problem by extracting the lecithin from egg noodles with hot absolute alcohol. This treatment presumably dissolved out the lecithin, kephalin, and possibly other phospholipins as well as certain additional substances including fat. Egg yolk being rich in phospholipin (10–15 per cent) and flour being relatively poor in this constituent, Juckenack was able to obtain progressive increases in the lecithin P_2O_5 with increases in the number of eggs per pound of flour.

Hertwig⁴, using the same general principle, devised a new and improved method in which a hot alcoholic extraction followed by ether was used. His data showed a greater percentage of lecithin P_2O_5 (or lipid P_2O_5 as he terms it) than could be obtained by the Juckenack method. Hertwig's method for lipid P_2O_5 determination in noodles and salad dressings was adopted tentatively by the A. O. A. C.

Rask and Phelps⁵ devised a method that differs from the Hertwig method in one particular only—an alkaline alcohol-ether extraction is made. Buchanan⁶, working on egg noodles, compared both methods of extraction and found good agreement in the percentage of lipid P_2O_5 recovered.

LECITHIN (LIPOID) PHOSPHORUS CONTENT OF EGGS.

It has long been known that egg yolk is rich in lecithin. Small amounts of other phospholipins also have been found, and as stated previously, food chemists have made use of this fact in devising methods for the determination of egg yolk in food products. Hertwig found commercial yolk to contain 1.78 per cent lipid P_2O_5 (dry basis) and the average value for dried whole egg solids to be 1.38 per cent. Values for the total solids content of fresh egg yolk reported in the literature and that obtained by the writer are as follows:

| | per cent |
|---------------------------------------|----------|
| Mojonnier and Troy ⁷ | 50.50 |
| Redfield ⁸ | 49.69 |
| Leach ⁹ | 49.50 |
| The writer (see Table 1)..... | 50.53 |
| Average..... | 50.05 |

¹ *Ber.*, 23, 423 (1890).

² *Analyst*, 15, 116 (1890).

³ *Z. Nahr. Genussm.*, 3, 13 (1900); 8, 94 (1904).

⁴ *This Journal*, 8, 116 (1924–5).

⁵ *Ibid.*, 109.

⁶ *Ibid.*, 7, 407 (1923–4).

⁷ *Technical Control of Dairy Products*, 1st ed., p. 288.

⁸ U. S. Dept. Agr. Bull. 846, p. 73.

⁹ *Food Inspection and Analysis*, 4th ed., p. 271.

To simplify the calculations which appear later in the paper, 50 per cent was adopted as a fair average value for total solids in fresh egg yolk. Analyses of the egg products used are compiled in Table 1. An average of all presumably unmodified egg yolk products, comprising laboratory numbers 561, 5734, 6137, 564, 696, and 849, in terms of milligrams of lipid phosphorus per 100 grams is 796.7. The figure obtained from Hertwig's average value for water-free commercial yolk (see above) is 777.7 mg. per 100 grams. The methods of analysis used were those proposed by Hertwig¹. The neutral extraction method for lipoids and lipid phosphorus was used except where otherwise designated. The results obtained by the writer are slightly higher than those obtained by Hertwig, but they serve to corroborate his results, which, it is assumed, were obtained from a fairly large number of samples.

TABLE 1.
Analyses of egg products.

| LAB. NO. | DESCRIPTION | TOTAL SOLIDS | LIPOIDS | LIPOID PHOSPHORUS | | LIPOID PHOSPHORUS (DRY BASIS) | PROTEIN (N × 6.25) |
|---|---|-----------------|-----------------|---------------------------|-----------------|-------------------------------------|-----------------------|
| | | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 558 | Fresh egg yolk separated from average - sized market eggs | | | 0 355 | 0.355 | | |
| 564 | Fresh egg yolk, prepared by separation of yolk from market eggs | 50.01 | 34.52 | 0 402 0 390 | 0 396 | 0 792† | . . . |
| 696 | “ “ “ “ | 51 11 | 33 09 | 0.413 0.418 | 0 416 | 0.814† | . . . |
| 849 | “ “ “ “ | 50.48 | 33 89 | 0.394 0 404* 0.407* | 0 402 | 0 797† | |
| 559 | Powdered egg yolk (?) from local bakery (possibly powdered whole egg) | | 53.38 | 0.524 0 529 | 0 527 | | 37.13 |
| 560 | Powdered egg yolk from local dairy store (not pure egg yolk) | 96.97 | 51.54 | 0.537 0.542 | 0.539 | 0.556 | 45 15 |
| 561 | Dried yolk prepared from market eggs | 96 75 | 67.11 | 0.795 0.764 | 0.779 | 0.805† | |
| 5734 | Dried egg yolk from local ice cream company | 93.23 | 61.57 | 0.715 | 0 715 | 0.767† | |
| 5831 | Commercial processed egg yolk powder | 94.98 | 45.46 | 0.516 | 0.516 | 0.543 | |
| 6137 | Commercial frozen egg yolk | 53.03 | 37.54 | 0.427 | 0.427 | 0.805† | |
| Average for pure yolk products (dry basis)..... | | | | | | 0.7967† | |

* Determined by Roese-Gottlieb (Mojonnier) method.

† Unadulterated egg yolk.

LIPOID PHOSPHORUS CONTENT OF CERTAIN DAIRY PRODUCTS.

It was found that while no values for the lipoid phosphorus content of ice cream were available in the literature, values for the "lecithin" content of milk, cream, skim milk, buttermilk, butter and other dairy products had been determined, but the methods used by the different investigators were not uniform. In a study of milk and milk products for the purpose of ascertaining the effect of lecithin on the butterfat determination, Chapman¹ reports the values for lecithin shown in Table 2.

TABLE 2.
Lecithin values obtained by Chapman.

| | FAT | LECITHIN | | | LIPOID PHOSPHORUS |
|------------------|-----------------|-----------------|-----------------|-----------------|-------------------|
| | | High | Low | Average | Calculated |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Cream. | 45 70 | 0 1824 | 0 2155 | 0.1981 | 0 00792 |
| Skim milk. | 0 153 | 0 0082 | 0 0290 | 0.0165 | 0.00066 |
| Buttermilk. | 0 643 | 0 1036 | 0.1480 | 0 1303 | 0.00521 |
| Milk ... | 3 848 | 0 0345 | 0 0709 | 0 0447 | 0 00179 |

Chapman also cites other investigators who give values for the lecithin content of whole milk shown in Table 3.

TABLE 3.
Lecithin values obtained by other investigators than Chapman.

| | LECITHIN | | LIPOID PHOSPHORUS |
|--------------------------|-----------------|-----------------|-------------------|
| | Average | | Calculated |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Stocklassa. | 0 09 - .113 | 0.1051 | 0 0042 |
| Burrow. | 0 049 - .1058 | 0 0535 | 0 0021 |
| Koch and Woods. | 0 072 - .086 | 0 0797 | 0.0032 |
| Nerking and Hansel. | 0 0364- 1163 | 0 0629 | 0.0025 |
| Glikin. | 0 0158- 1173 | 0 0765 | 0 0031 |
| Averages. | | 0.0755 | 0 0030 |

In Tables 2 and 3 the writer added the column containing the calculated lipoid phosphorus; these results were computed on the generally accepted basis that lecithin contains 4 per cent phosphorus. Cusick² found 0.0432 per cent (0.00173 per cent P) to 0.0723 per cent (0.00289 per cent P) lecithin in butter. Bordas and de Raczkowski³ found 0.334 per cent lecithin (0.0133 per cent P) in 50.88 per cent cream. Dornic

¹ *J. Dairy Sci.*, 11, 429-45 (1928).

² Reported by Supplee, Cornell Univ. Agr. Expt. Sta. Memoir 29, p. 110 (1919).

³ *Comp. rend.*, 135, 302 (1902).

and Daire¹ found lecithin in raw cream to the extent of 0.0905 per cent (0.00362 per cent P) to 0.0944 per cent (0.00378 per cent P). It is apparent that the lipid phosphorus content of milk and milk products ascertained by these investigators is small.

In order to extend and amplify the results cited, several samples of commercial ice cream mix were analyzed primarily for lipid phosphorus. Many samples of various dairy products used in the manufacture of ice cream were also analyzed. A uniform technic, which was perfected after extensive experimental work (see Part II), was used for the determination of lipid phosphorus. The results of these analyses are briefly summarized in Table 4.

TABLE 4.

Comparison of average lipid phosphorus and fat content of commercial ice cream mix and of several dairy products used in their manufacture.

| DESCRIPTION | NO. OF SAMPLES ANALYZED | FAT <i>average per cent</i> | LIPOID PHOSPHORUS | | |
|------------------------------------|-------------------------------|--|--------------------------|---------|---------|
| | | | Maximum | Minimum | Average |
| | | | <i>mg. per 100 grams</i> | | |
| Commercial ice cream mix | 21 | 11.54 | 3.09 | 1.78 | 2.56 |
| Special ice cream mix | 6 | 18.83 | 4.71 | 2.97 | 3.84 |
| Cream | 7 | 39.19 | 8.54 | 4.91 | 6.84 |
| Skim milk powder | 6 | 1.09 | 5.57 | 4.66 | 5.17 |
| Condensed skim milk | 6 | 0.43 | 2.35 | 1.28 | 1.76 |
| Pasteurized market milk | 4 | 4.54 | 1.16 | 1.08 | 1.11 |
| Partially skimmed milk | 1 | 1.80 | . . | . . | 0.81 |
| Skim milk | 1 | 0.13 | . . | . . | 0.53 |
| Sweet butter | 7 | 80.85 | 7.65 | 3.98 | 5.58 |
| Butter fat | 1 | 100.0 | . . | . . | None |

The lipid phosphorus content of commercial ice cream mix of average or common composition was shown to be relatively constant in amount. The fact that it is not consistently dependent upon nor related to the fat content (see Refs. 6 and 7, p. 279) of the dairy products is strikingly brought out by a comparison of these values for commercial ice cream mix and condensed skim milk or skim milk powder.

The average value for 21 commercial mixes of from 10–15 per cent fat content is 2.56 mg. per 100 grams, while that for condensed skim milk is 1.76 mg., and for skim milk powder (fat content approximately 1 per cent), 5.17 mg. per 100 grams. A fair proportionality of lipid phosphorus exists between whole milk (average value 1.11) and commercial evaporated milk (value 2.95), as well as between skim milk, condensed skim and powdered skim. Furthermore, these observations tend strongly to show that lipid phosphorus is not lost to any appreciable extent upon evaporation, sterilization or drying of these products, despite statements to be found in the literature relative to the destruction or hydrolysis of lecithin.

This brings up the question of whether or not additional basic lipid

¹ *Ann. fals.*, 30, 533 (1910).

phosphorus values should be established for ice cream mixes of high fat content in order to procure greater accuracy in the application of formulas. As brought out by an inspection of the lipid phosphorus values for sweet cream samples and certain high fat content mixes analyzed, an increase in this value for the mix is to be expected theoretically if the fat content is very materially increased by the addition of sweet cream (see below). The lipid phosphorus values for the experimental high fat content mixes are practically equivalent to the theoretical amounts to be expected from the known composition of the major constituents. This may be made clear by the following considerations: Since the lipid phosphorus value for the egg-free base mix was found to be 2.55 and that for the sweet cream 8.54, then the theoretical yield of lipid phosphorus in any admixture of the two may be calculated. The mixtures with corresponding theoretical and actual lipid phosphorus values expressed in milligrams per 100 grams are as follows:

| MIXTURE | SWEET CREAM | | BASE MIX | | TOTAL L. P. (THEORY) | ACTUAL L. P. (BY ANALYSIS) |
|---------|------------------|------------------|------------------|------------------|-------------------------|-------------------------------|
| | Per cent used | L. P. content | Per cent used | L. P. content | | |
| 1 | 23.1 | 1.97 | 76.9 | 1.95 | 3.92 | 4.02 |
| 2 | 39.4 | 3.36 | 60.6 | 1.55 | 4.91 | 4.71 |

The type of commercial mix commonly found in California is one composed of milk, cream, condensed skim milk, gelatin, sugar and flavoring. A typical composition of such a mix would be as follows:

| | pounds | | LIPID PHOSPHORUS mg. per 100 grams |
|-----------------------------|--------|----------|---------------------------------------|
| Milk..... | 45.00 | yielding | 0.50 |
| Cream, 37.5 per cent fat .. | 25.00 | " | 1.71 |
| Condensed skim milk..... | 16.00 | " | 0.28 |
| Sugar..... | 14.00 | " | |
| Gelatin..... | 0.35 | " | |
| Totals..... | 100.35 | | 2.49 |

The above calculations of lipid phosphorus content of such a mix are based on average values obtained by the writer for these constituents (see Table 4). The theoretical amount, 2.49 mg. per 100 grams, is not far from the average of 21 commercial egg-free mixes analyzed (2.56 mg. per 100 grams).

Another type of mix commonly met with has the following general composition:

| | pounds | | LIPID PHOSPHORUS mg. per 100 grams |
|----------------------------|--------|----------|---------------------------------------|
| Milk (4 per cent fat)..... | 55.00 | yielding | 0.61 |
| Cream (40 per cent fat)... | 24.50 | " | 1.51 |
| Skim milk powder..... | 4.48 | " | 0.23 |
| Sugar..... | 14.00 | " | |
| Gelatin..... | 0.35 | " | |
| Water..... | 1.67 | " | |
| Totals..... | 100.00 | | 2.35 |

TABLE 5.

Tabulation of percentage composition by ingredients of eleven ice cream mixes; also lipid phosphorus content predicted by calculation and found by analysis.

| LAB. NO. | CREAM, APPROX. 40% | MILK, AVERAGE 2.8% FAT | SKIM MILK | CONDENSED SKIM | SKIM POWDER | BUTTER | SUGAR | GELATIN | WATER | TOTAL L. P. CALCULATED (MG. PER 100 GRAMS) | L. P. BY ANALYSIS (MG. PER 100 GRAMS) | FAT |
|---------------|--------------------|------------------------|-----------|----------------|-------------|--------|-------|---------|-------|--|---------------------------------------|-------|
| 2087 | 32.47 | 41.23 | | 10.00 | | | 15.75 | .49 | | 2.85 | 3.07 | 12.70 |
| 2100 | 49.00 | | 35.00 | | | | 15.00 | .20 | | 3.54 | 3.83 | 18.48 |
| 2104 | 21.40 | | 37.40 | 23.40 | | 3.60 | 14.60 | .48 | | 2.28 | 2.44 | 11.41 |
| 2214 | 35.10 | | 27.30 | 19.70 | | | 15.00 | .41 | 2.60 | 2.89 | 2.92 | 13.79 |
| 2682 | 4.90 | | | 22.00 | 3.30 | 11.00 | 16.00 | .50 | 41.80 | 1.51 | 1.77 | 11.07 |
| 2689 | 21.40 | | | 20.40 | 3.50 | 1.60 | 16.20 | .49 | 36.10 | 2.09 | 2.30 | 10.07 |
| 2690 | 34.80 | | 27.80 | 21.80 | | | 15.00 | .30 | | 2.86 | 3.04 | 13.83 |
| 3587 | 42.00 | 43.00 | | | | | 15.00 | .20 | | 3.35 | 4.15 | 18.33 |
| 3588 | 16.00 | | 40.00 | 24.00 | | 6.00 | 15.00 | ... | | 2.06 | 2.37 | 10.58 |
| 3589 | 22.00 | | 40.00 | 17.00 | | 5.00 | 15.00 | .50 | | 2.30 | 2.75 | 14.26 |
| 3590 | 5.00 | | 42.00 | 21.00 | | 12.00 | 16.00 | ... | 4.00 | 1.60 | 2.17 | 10.52 |
| Averages..... | | | | | | | | | | 2.485 | 2.801 | |

The theoretical lipid phosphorus content of this mix, like the one preceding, is practically equivalent to the average value obtained for egg-free mix previously cited.

In compounding the experimental high-fat mixes above referred to, the increase in the lipid phosphorus of these mixes over that of the base mix from which they were prepared, is roughly proportional to the fat content and bears a striking proportional relationship to the amount of cream used, which is the source of the greater part of the lipid phosphorus¹.

In this connection it is of interest to refer to Table 5, which shows the extent to which, or the accuracy with which, the lipid phosphorus content of various egg-free commercial mixes has been predicted by calculations based on the percentage composition of the mix by ingredients. These calculated values were determined from the average values for the lipid phosphorus content of the various constituents as obtained, in most cases, by an appreciable number of analyses of representative products (see Table 4).

By way of probable explanation of these observations it may be tentatively assumed that the lipid phosphorus fraction is intimately associated with the membranes surrounding the fat globules. This belief seems to be borne out by the researches of Bordas and de Raczkowski², Bischoff³, Thurston and Peterson⁴, and Dornic and Daire⁵, all of whom found that the lecithin content of skim milk and buttermilk was very much greater in proportion to the fat content than was true of whole milk, cream or butter. It has been reported by Osborne and Wakeman⁶, that butterfat itself contains no lecithin. This has been confirmed by the writer (see Table 4). The "Slimmen-Membran" of Storch, which disengages itself in the churning of cream into butter, was found by Palmer and Samuelson⁷ to be composed very largely of phospholipins. It seems logical to suppose that the chief reason for the proportionally higher lecithin content of the fatty matter in skim milk may be due to phospholipins occurring associated with the relatively large number of extremely small fat globules contained therein. Bordas and de Raczkowski report 0.018 per cent lecithin in skim milk that contains 0.09 per cent fatty material, or 20 per cent.

DEVELOPMENT OF FORMULAS.

For the estimation of egg solids in noodles, Hertwig⁸ developed for-

¹ This observation may appear to be a contradiction of the statement made previously, but the reader is referred to Refs. 6 and 7, in the hope that these views may become reconciled.

² Loc. cit.

³ *Z. physiol. Chem.*, 173, 227 (1928).

⁴ *J. Dairy Sci.*, 11, 270 (1928).

⁵ Loc. cit.

⁶ Cited by Rogers et al., *Fundamentals of Dairy Science*, p. 72.

⁷ *Proc. Soc. Exp. Biol. Med.*, 21, 537 (1924).

⁸ *Methods of Analysis*, A. O. A. C., 1925, 234.

mulas which are based upon the average values obtained for the lipid P_2O_5 in whole egg and egg yolk solids, together with the average value determined for the lipid P_2O_5 content of flour and semolina.

These formulas were applied by Buchanan, and the results of her investigations on the estimation of egg solids content of noodles of known composition showed that they give very close approximations of the actual whole egg and yolk solids content in the material studied.

On the basis of the success obtained by Hertwig and Buchanan in devising and applying a procedure and formulas for the estimation of egg solids in noodles, the writer proposed to utilize their methods in part, at least, as a tentative procedure in the solution of the problem involving the quantitative determination of egg solids, particularly egg yolk, in ice cream. For egg yolk the average value proposed by Hertwig for the lipid P_2O_5 content of yolk solids (expressed in terms of phosphorus) was adopted. The average lipid phosphorus value for commercial ice cream (2.56 mg. per 100 grams), given previously, was used as a basis for calculations. The formula for dry egg yolk, therefore, becomes:

$$E = \frac{(A - 2.56) \times 100}{777.7 - 2.56} = \text{percentage of dry egg yolk; or}$$

$$E = (A - 2.56) \times .129, \text{ in which}$$

$$E = \% \text{ dry egg yolk, and}$$

$$A = \text{lipoid phosphorus in sample expressed in mg. per 100 grams.}$$

777.7 = mg. phosphorus per 100 grams dry egg yolk, based on Hertwig's value for lipid P_2O_5 in commercial yolk (1.78 per cent). No factor was applied for losses of lipid phosphorus. It has been found on the average that approximately 96 per cent recovery of the lipid phosphorus is obtained.

A formula for whole egg solids may be easily derived from the above by using Hertwig's basic value for the lipid P_2O_5 percentage in whole egg (1.38 per cent P_2O_5 or 0.6028 per cent phosphorus), thus changing the constant factor from 0.129 to 0.166. Therefore, for whole egg solids the formula becomes: $E = (A - 2.56) \times 0.166$. In converting dry egg yolk percentages into fresh egg yolk the former is multiplied by two (see above).

PART II.

EXPERIMENTAL TECHNIC.

After a number of preliminary trials, the well-known Roese-Gottlieb (Mojonnier) extraction with minor modifications was adopted as an accurate and rapid means of obtaining the total lipid material from milk products. Since only the phosphorus content was to be considered,

the residue left upon the evaporation of the extract was subsequently treated to ascertain the exact amount of this element present. A considerable amount of experimental work was carried out by using different methods for the colorimetric phosphorus determination as applied to organic materials, particularly lipoids.

The method proposed by Roe, Irish, and Boyd¹, that devised by Harnes² based upon the published work of Briggs³ and of Roe, Irish and Boyd, and the method of Benedict and Theiss⁴ were tried in this work. For one reason or another, none of these procedures was found satisfactory, but after many trials with them and with various modifications, the following procedure was adopted:

The Roese-Gottlieb (Mojonnier) method, as well as a slight modification employing heat after the addition of the water, ammonia and alcohol, was used for the extraction of the total lipoids. Two extractions of 25 cc. each of ethyl and petroleum ether were made. After evaporation of the ether the residue was dried in a water oven, taken up with dry chloroform and filtered through cotton placed in the stem of a small funnel. This solution was made up to 50 cc. with chloroform or in some cases with 95 per cent alcohol in order to economize on chloroform consumption.

A suitable aliquot was placed in a small flat-bottomed platinum dish. One cc. of magnesium nitrate (50 per cent solution) was added, and the solution was evaporated to dryness on the steam bath. A few drops of concentrated nitric acid were added, and the dish and contents were then gently ignited, care being exercised that the oxidation did not proceed too rapidly and thus cause loss of residue from the dish. Finally, the residue was strongly ignited to remove all carbon and any traces of NO₂ remaining. The residue, which should be perfectly white, was next taken up with a small amount of distilled water and 2 cc. of sulfuric acid (1 + 1) was added. Complete solution was effected and facilitated by agitation of the material with a small stirring rod and by heating on the water bath. The solution, which should be perfectly clear, was then transferred to a 200 mm. test tube calibrated with a mark at 20 cc. The dish was washed several times with small quantities of distilled water, and the volume was finally made up to 20 cc. The reagents were then added as follows: 2 cc. of sodium bisulfite (15 per cent) containing 0.5 per cent hydroquinone followed by 2 cc. of ammonium molybdate, according to Briggs⁵. The mixture in the test tube was then shaken, after which it was placed in a boiling water bath for 15 minutes. A clear blue color was produced. The tubes were then cooled in running water.

¹ *J. Biol. Chem.*, 67, 579 (1926).

² *Ibid.*, 77, 406 (1928).

³ *Ibid.*, 53, 13 (1922).

⁴ *Ibid.*, 61, 63 (1924).

⁵ *Loc. cit.* C. P. ammonium molybdate (25 grams) in 300 cc. of H₂O plus 200 cc. of dilute H₂SO₄ (125 cc. of H₂O plus 75 cc. of concentrated H₂SO₄).

TABLE 6.

Results of duplicate analyses of ice cream and other dairy products (expressed in mg. per 100 grams of lipid phosphorus).

| LAB. NO. | A | B | C | AVERAGE | REMARKS |
|----------|-------|-------|------|---------|--------------------------|
| 5385 | 2.77 | 2.80 | | | Mix |
| 5385-B | 7.35 | 7.17 | | | Mix |
| 6087 | 2.65 | 2.67 | 2.61 | 2.64 | Mix |
| 6087-A | 5.73 | 5.95 | | 5.84 | Mix |
| 562 | 2.65 | 2.58 | | 2.62 | Mix |
| 563 | 2.55 | 2.48 | | 2.52 | Mix |
| 563-B | 13.54 | 14.02 | | 13.78 | Mix |
| 695 | 2.43 | 2.87 | | 2.65 | Mix |
| 695-A | 20.71 | 20.77 | | 20.74 | Mix |
| 848 | 2.51 | 2.66 | 2.49 | 2.55 | Mix |
| 848-A | 18.10 | 18.20 | | 18.15 | Mix |
| 848-B | 7.04 | 7.15 | | 7.10 | Mix |
| 848-C | 31.37 | 31.66 | | 31.52 | Mix |
| 886 | 4.03 | 4.01 | | 4.02 | Mix |
| 887 | 4.84 | 4.58 | | 4.71 | Mix |
| 1829 | 3.37 | 3.37 | | 3.37 | Mix |
| 1830 | 2.31 | 2.47 | | 2.39 | Mix |
| 1831 | 2.89 | 3.05 | | 2.97 | Mix |
| 1908 | {2.58 | {2.56 | | 2.64 | Mix |
| | {2.80 | {2.61 | | | |
| 1941 | 2.68 | 2.82 | | 2.75 | Mix |
| 1943 | 2.45 | 2.40 | | 2.43 | Mix |
| 2087 | 3.07 | 3.11 | | 3.09 | Mix |
| 2088 | 4.61 | 4.67 | | 4.64 | Mix |
| 2100 | 3.86 | 3.79 | | 3.83 | Mix |
| 2104 | 2.86 | 2.88 | | 2.87 | Mix |
| 2214 | 2.89 | 2.94 | | 2.92 | Mix |
| 2682 | 1.73 | 1.83 | | 1.78 | Mix |
| 3587 | 4.24 | 4.07 | | 4.15 | Mix |
| 3888 | 2.32 | 2.43 | | 2.37 | Mix |
| 3589 | 2.69 | 2.82 | | 2.75 | Mix |
| 3590 | 2.03 | 2.32 | | 2.17 | Mix |
| 1904 | 5.13 | 5.15 | | 5.14 | Past. sweet cream |
| 1906 | 7.78 | 7.42 | | 7.60 | Past. sweet cream |
| 2102 | 7.30 | 7.14 | | 7.22 | Past. sweet cream |
| 1903 | 3.83 | 4.13 | | 3.98 | Sweet butter |
| 1942 | 5.99 | 6.46 | | 6.23 | Sweet butter |
| 2106 | 7.60 | 7.71 | | 7.65 | Sweet butter |
| 1905 | 2.19 | 2.52 | | 2.35 | Condensed skim milk |
| 1940 | 2.19 | 2.12 | | 2.16 | Sweetened condensed skim |
| 2105 | 1.31 | 1.25 | | 1.28 | Condensed skim milk |
| 2683 | 1.54 | 1.69 | | 1.62 | Condensed skim milk |
| 1900 | 4.95 | 5.19 | | 5.07 | Dried skim milk |
| 1901 | 5.52 | 5.58 | | 5.55 | Dried skim milk |
| 1907 | 4.93 | 5.12 | | 5.03 | Dried skim milk |

and the solution was made to a definite volume with distilled water preparatory to comparison with standards in a colorimeter. Standards were prepared by treating definite small volumes of a standard potassium dihydrogen phosphate solution (0.2193 gram per liter), in a manner similar to that employed in the test. One cc. of this solution was equivalent to 0.05 mg. of phosphorus. This standard stock solution was preserved by adding a few drops of chloroform. It was found that color values produced by 0.05–0.10 mg. of phosphorus were most satisfactory to work with. Thus, standard color solutions were prepared by subject-

ing 1-2 cc. of the standard phosphate solution to the procedure above outlined. It was found that the oxidation of the standard with magnesium nitrate was not necessary in order to produce true comparative colors. Repeated experiments proved that the colors developed by a given amount of phosphate solution were practically identical whether or not the treatment with magnesium nitrate was employed.

This procedure was subjected to critical study and found to be reliable and accurate. Results could be duplicated within reasonable limits of experimental error (see Table 6).

GENERAL PLAN OF WORK AND APPLICATION OF FORMULA.

After the development of a technic which appeared to be satisfactory, work was begun upon the examination of ice cream mix, several dairy products which are common constituents thereof, and eggs and egg products. The lipid phosphorus content of a number of egg-free mixes, as well as of milk, skim milk, condensed skim, condensed milk, cream and butter was determined.

Samples of egg-free ice cream mix were analyzed for fat (total lipoids) and lipid phosphorus. To some of these samples varying amounts of fresh egg yolk, yolk powder, etc., were added, and the lipid phosphorus was again determined; the percentage recovery of this constituent was determined and an attempt was made to apply the formula for the calculation of egg yolk present on the basis of the data obtained, together with data available in the literature especially with respect to the lipid phosphorus content of egg yolk.

In Table 7 the analyses of several ice cream mixes are given, as well as the analyses of the egg products used in compounding the various mixtures examined. An examination of the data will disclose the fact that the percentages of egg yolk have been very closely approximated by the use of the formula. There was an average percentage recovery of lipid phosphorus of 95.51 per cent, a minimum of 88.08 per cent and a maximum of 103.3 per cent. The average percentages of dry yolk and of fresh yolk actually present compare very favorably with percentages determined by formula.

EFFECT OF USING HEAT ON THE ROESE-GOTTLIEB EXTRACTION.

In Table 8 the percentages of egg yolk obtained by formula in samples of evaporated milk and ice cream mix, which were admixed with known amounts of egg yolk, are presented. A limited amount of comparative data was obtained on these samples by both the regular Roese-Gottlieb (Mojonnier) extraction, and by the modified Mojonnier method in which the first extraction was accompanied by the application of heat after the addition of the water, ammonia, and alcohol. In these latter cases the mixture was kept at the boiling point for 2 minutes before the ether

TABLE

Recovery of lipid phosphorus added to ice cream mix in form of fresh egg yolk—

NOTE.—Average percentage of fresh egg yolk

| LAB. NO. | DESCRIPTION | FAT AND LIPOIDS per cent | LIPOID PHOSPHORUS (MG. PER 100 GRAMS) | | |
|--|---|--------------------------------|--|---------|--------|
| | | | Found | Average | Added |
| 562 | Ice cream mix, egg free, "A" Ice Cream Co. | | 2.65 2.58 | 2.62 | |
| 562-A | Ditto, containing 1% fresh egg yolk, No. 564 | 12.86 | 6.48 | 6.48 | 3.96 |
| 562-B | Ditto, containing 2.91% fresh egg yolk, No. 564 | 13.24 | 14.13 | 14.13 | 11.52 |
| 563 | Ice cream mix, egg yolk-free "B" Dairy Co. | | 2.55 2.48 | 2.52 | |
| 563-A | Ditto, containing 1% fresh egg yolk, No. 564 | 11.34 | 6.46 | 6.46 | 3.96 |
| 563-B | Ditto, containing 2.91% fresh egg yolk, No. 564 | 11.87 | 13.54 14.02 | 13.78 | 11.52 |
| 564 | Fresh egg yolk prepared by separation of yolks from market eggs | 34.52 | 402.0 389.3 | 395.6 | |
| 695 | Ice cream mix, egg-free, "A" Ice Cream Co. | 11.14 | | 2.65 | |
| 695-A | Ditto, containing 4.83% fresh egg yolk, No. 696 | 12.27 | 20.71 | 20.71 | 20.06 |
| 695-B | Ditto, containing 1.12% fresh egg yolk, No. 696 | 11.26 | 6.43 | 6.43 | 4.65 |
| 695-C | Ice cream mix No. 695, containing 7.92% fresh egg yolk, No. 696 | 13.00 | 31.41 | 31.41 | 32.88 |
| 696 | Fresh egg yolk prepared by separation of yolks of market eggs | 33.09 | 412.7 418.0 | 415.3 | |
| 695-D | Ice cream mix No. 695, containing 1% fresh egg yolk, No. 696 | 11.50 | 7.02 6.05 | 6.54 | 4.15 |
| 695-D | Ditto, after pasteurization for 40 minutes at 145°F. | 11.52 | 6.64 | 6.64 | 4.15 |
| 695-E | Ice cream mix No. 695, containing 3% fresh egg yolk, No. 696 | 11.95 | 13.57 | 13.57 | 12.46 |
| 695-E | Ditto, after pasteurization for 40 minutes at 145°F. | 11.98 | 14.01 | 14.01 | 12.46 |
| 695-F | Ice cream mix No. 695, containing 5% fresh egg yolk, No. 696 | 12.44 | 22.94 | 22.94 | 20.77 |
| 695-F | Ditto, after pasteurization for 40 minutes at 145°F. | 12.43 | 21.74 | 21.74 | 20.77 |
| 695 | Base sample (mix), no egg, after pasteuri- zation for 40 minutes at 145°F. | 11.25 | 2.73 | 2.73 | .. |
| 848 | Experimental egg-free mix, prepared in laboratory from milk, cream, condensed skim, sugar and gelatin | 11.07 | 2.51 2.66 2.49 | 2.55 | |
| 848-A | Mix No. 848, containing 3.83% fresh egg yolk, No. 849 | 11.84 | 18.10 18.20 | 18.15 | 15.40 |
| 848-B | Mix No. 848, containing 1.14% fresh egg yolk, No. 849 | 11.07 | 7.04 7.15 | 7.10 | 4.58 |
| 848-C | Mix No. 848, containing 7.55% fresh egg yolk, No. 849 | 12.54 | 31.37 31.66 | 31.52 | 30.36 |
| 849 | Fresh egg yolk prepared by separation of yolks from market eggs | | 394.0 404.6 407.7 | 402.1 | |
| Averages of all egg mixes (16 in all)..... | | | | 15.100 | 13.353 |

* Differences equal figures to be added to or subtracted from percentages found by formula to equal actual percentages of egg yolk.

7.

percentages of egg yolk calculated by formula based on lipid phosphorus content.
actually present in samples, 3.263.

| RECOVERY OF LIPOID PHOSPHORUS | | CALCULATED EGG YOLK— BY FORMULA | | DRY EGG YOLK ACTUALLY PRESENT | DIFFERENCES BETWEEN ACTUAL AND CALCULATED EGG YOLK* | |
|-------------------------------------|-------|------------------------------------|--------------------------|-------------------------------------|---|------------------------|
| <i>per cent</i> | | Dry <i>per cent</i> | Fresh <i>per cent</i> | <i>per cent</i> | Fresh <i>per cent</i> | Dry <i>per cent</i> |
| | | | . . . | | | |
| 98.50 | | 0.50 | 1.00 | 0.50 | 0.0 | 0.0 |
| 99.93 | | 1.49 | 2.98 | 1.46 | -0.07 | -0.03 |
| | | . . | . . | . . | | . . . |
| 99.67 | | 0.50 | 1.00 | 0.50 | 0.0 | 0.0 |
| 98.15 | | 1.44 | 2.88 | 1.46 | +0.03 | +0.02 |
| | | | . | 50.01 | | |
| | | . . | . | . | | . . . |
| 91.20 | | 2.34 | 4.68 | 2.47 | +0.15 | +0.13 |
| 88.08 | | 0.50 | 1.00 | 0.57 | +0.12 | +0.07 |
| 88.36 | | 3.72 | 7.44 | 4.05 | +0.48 | +0.33 |
| | | . . | . . | 51.11 | . . . | . . |
| 103.30 | Av. | 0.57 | 1.14 | | | |
| 90.30 | 96.80 | 0.45 | 0.90 | 0.51 | -0.02 | 0.0 |
| | | 0.51 | 1.02 | | | |
| 97.65 | | 0.52 | 1.04 | 0.51 | -0.04 | -0.10 |
| 89.81 | | 1.43 | 2.84 | 1.53 | +0.16 | +0.10 |
| 92.72 | | 1.48 | 2.96 | 1.53 | +0.04 | +0.05 |
| 97.95 | | 2.63 | 5.26 | 2.55 | -0.26 | -0.08 |
| 92.83 | | 2.47 | 4.94 | 2.55 | +0.06 | +0.08 |
| | | . . | . | . | | |
| | | . . | . . . | . . . | | |
| 101.10 | | 2.01 | 4.02 | 1.93 | -0.19 | -0.08 |
| 99.58 | | 0.58 | 1.16 | 0.58 | -0.02 | 0.0 |
| 95.78 | | 3.73 | 7.46 | 3.81 | +0.09 | +0.08 |
| | | . . | . . | 50.48 | | . . . |
| 95.507 | | 1.616 | 3.230 | 1.657 | +0.034 | +0.041 |

TABLE 8.

Recovery of lipid phosphorus added in the form of egg yolk to evaporated milk and to ice cream mix—comparison of regular Rose-Gottlieb method and modification of same.

| LAB. NO. | DESCRIPTION | LIPID PHOSPHORUS (MG. PER 100 GRAMS) | | | | | | | | | | EGG YOLK ACTUALLY PRESENT (DRY BASIS) | EGG YOLK CALCULATED BY FORMULA (DRY BASIS) | | |
|----------|--|--------------------------------------|----------|--------------|----------|--------|--------------|--------------|-----------|-----------|----------|---------------------------------------|--|--------|--------|
| | | FOUND | | | | ADDED | RECOVERED | | Reg. Moj. | Mod. Moj. | | | | | |
| | | By Reg. Moj. | | By Mod. Moj. | | | By Reg. Moj. | By Mod. Moj. | | | | | | | |
| | | per cent | per cent | per cent | per cent | | | | | | per cent | | per cent | | |
| 5385 | Evaporated milk | 2.77 | 2.79 | 3.03 | 2.95 | | | | | | | | | | |
| 5385-A | Evaporated milk, No. 5385, with 1.5% commercial yolk powder | 2.80 | 2.79 | 2.86 | 2.86 | | | | | | | | | | |
| 5385-B | Evaporated milk, No. 5385, with 0.64% commercial yolk powder | | 13.05 | | | 10.73 | 96.52 | 92.03 | 92.03 | 96.52 | 92.03 | 1.40 | 1.35 | 1.29 | 1.29 |
| 5734 | Commercial yolk powder | | 7.35 | .. | 7.17 | 4.58 | 99.73 | 95.22 | 95.22 | 99.73 | 95.22 | 0.59 | 0.61 | 0.59 | 0.59 |
| 6087 | Ice cream mix; no egg | | | 2.53 | 715.2* | .. | | | | | | 93.23 | | .. | .. |
| 6087-A | Ice cream mix, No. 6087, with 0.5% commercial yolk powder | | 2.65 | 2.61 | 2.60 | .. | | .. | .. | | .. | | .. | .. | .. |
| 6087-B | Ice cream mix, No. 6087, with 1% commercial yolk powder | .. | | .. | 5.84 | 3.58 | | 94.56 | 94.56 | | | 0.47 | | | 0.43 |
| 848 | Ice cream mix; no egg | | | 2.66 | 9.23 | 7.15 | .. | .. | .. | .. | .. | 0.93 | .. | .. | 0.86 |
| 849 | Fresh egg yolk | | 2.51 | 2.49 | 2.58 | .. | | | | | | 50.48 | | | |
| 848-A | Ice cream mix, No. 848, with 3.83% fresh egg yolk | | 404.6 | .. | 407.7 | | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 848-B | Ice cream mix, No. 848, with 1.14% fresh egg yolk | .. | 18.10 | .. | 18.20 | 15.40 | 101.1 | 101.2 | 101.2 | 101.1 | 101.2 | 1.93 | 2.01 | 2.02 | 2.02 |
| 848-C | Ice cream mix, No. 848, with 7.55% fresh egg yolk | ... | 7.04 | .. | 7.15 | 4.58 | 99.3 | 99.8 | 99.8 | 99.3 | 99.8 | 0.58 | 0.57 | 0.59 | 0.59 |
| 886 | Experimental mix; no egg | | 31.37 | | 31.66 | 30.36 | 95.32 | 96.20 | 96.20 | 95.32 | 96.20 | 3.81 | 3.72 | 3.75 | 3.75 |
| 887 | Experimental mix; no egg | | 4.03 | | 4.01 | .. | .. | .. | .. | .. | .. | | | | |
| | | | 4.84 | | 4.58 | .. | .. | .. | .. | .. | .. | | | | |
| | Averages† | 9.373† | 9.373† | 9.349† | 9.349† | 9.349† | 98.52‡ | 96.89§ | 96.89§ | 98.52‡ | 96.89§ | 1.662§ | 1.652§ | 1.648§ | 1.648§ |

* Hertwig neutral extraction method used.

† Values for 5734 and 849 omitted from averages.

‡ Average of 10 samples.

§ Average of 5 samples.

extractions were made. No significant or consistent differences in the results obtained by these procedures were observed. It seems reasonably certain, therefore, that a hot extraction is not necessary to free the lipoids from the substances associated with them. The average amounts of lipid phosphorus found by the regular Mojonnier and by the modified Mojonnier methods are practically identical, as are the average calculated amounts of egg yolk found.

RECOVERY OF YOLK POWDER FROM MIX.

The calculated percentages of egg yolk and the percentages of recovery of lipid phosphorus added to egg-free mix in the form of commercial yolk powder appear in Table 9. A brief examination of these figures will show that the percentages of egg yolk obtained by formula are approximately equivalent to the amounts actually present.

TABLE 9.

Calculated percentages of egg yolk and the percentage of recovery of lipid phosphorus added to egg-free mix in form of dry egg yolk.

| LAB. NO. | DESCRIPTION | LIPOID PHOSPHORUS (MG PER 100 GRAMS) | | LIPOID PHOSPHORUS RECOVERY | EGG YOLK (DRY BASIS) CALCULATED BY FORMULA | EGG YOLK ACTUALLY PRESENT (DRY BASIS) | DIFFERENCE* |
|----------|--|---|-------|----------------------------------|---|--|-------------|
| | | Found | Added | | | | |
| 562 | Egg-free mix | 2 62 | | | | | |
| 563 | Egg-free mix | 2 32 | | | | | |
| 562 | Contains 0.25% com'l yolk powder | 4 33 | 1 788 | 98 18 | 0 22 | 0 23 | +0 01 |
| 562 | Ditto—1.96% | 15 78 | 14 03 | 94 78 | 1 70 | 1 83 | +0.13 |
| 563 | Ditto—0.25% | 4 18 | 1 788 | 97 02 | 0 21 | 0 23 | +0 02 |
| 563 | Ditto—1.96% | 14 68 | 14 03 | 88 77 | 1 56 | 1 83 | +0 27 |
| 5734 | Com'l yolk powder used | 715 2 | | | | 93 23 | |
| | | 2 55) | | | | | |
| 6087 | Egg-free mix | 2 61 } 2 60 | | | | | |
| | | 2 67) | | | | | |
| 6087A | Containing 0.5% egg yolk powder | 5 84 | 3 576 | 94 56 | 0 43 | 0 47 | +0 04 |
| 6087B | Ditto—1.0% | 9 23 | 7 152 | 94 67 | 0 86 | 0 93 | +0 07 |
| | Average values for egg mix | 9 007 | 7 056 | 94 66 | 0 83 | 0 92 | +0 09 |

* Differences equal figures to be added to or subtracted from the percentages found by formula to equal the actual percentages of egg yolk.

EFFECT OF AGING OF SAMPLE.

McLean¹ states that lecithin is very easily oxidized and that great precautions must be taken in its isolation and purification. He also cites the researches of Bokay who investigated the action of lipase on

¹ Lecithin and Allied Substances, The Lipins, 2nd ed., pp. 17 and 37.

lecithin and found that it was split into glycerophosphoric acid, choline and fatty acids by this enzyme. Bordas and de Raczkowski¹ state that the lecithins of milk are partially destroyed by heat; they found definite and progressive losses ranging from 14 to 30 per cent when milk was heated from 60°–110°C. for 30 minutes. In a study of the lecithin content of butter and its relation to the fishy flavor, Supplee² contends that this flavor is due to the partial hydrolysis of lecithin. Sommer and Smit³ made an elaborate study of the fishy flavor of butter, cited many authorities, and submitted much experimental evidence of the decomposition of lecithin in salted butter as a cause of fishy flavor. They indicate that this decomposition is accomplished by a combined hydrolysis and oxidation of the lecithin with the production ultimately of trimethyl amine and other compounds.

In view of these findings it was thought desirable to ascertain the effect of storage or of holding the sample upon the recovery of lipid phosphorus. Both egg-free ice cream mix and the same containing different percentages of egg yolk were used in the experiment. A limited study of this problem is presented in Table 10. The samples, after the initial analyses, were held in the refrigerator at from 2° to 5°C., for varying lengths of time; in some cases as much as one month elapsed before the lipid phosphorus content was again determined. At the time of the second analysis the samples were sour, showed unmistakable evidence of partial decomposition, and possessed a very stale odor. The recovery of lipid phosphorus from these samples is shown in the table. Very slight losses in lipid phosphorus were obtained, so slight in fact as to fall practically within the range of experimental error.

TABLE 10.

Lipid phosphorus content of ice cream mix before and after storage in refrigerator (mg. phosphorus per 100 grams), no preservative used.

| LAB. NO. | EGG YOLK CONTENT (DRY BASIS) | DATE ANALYZED FRESH | LIPOID PHOSPHORUS mg. | DATE ANALYZED AFTER STORAGE | LIPOID PHOSPHORUS mg. |
|---------------------|------------------------------------|------------------------|-----------------------------|--------------------------------|-----------------------------|
| | per cent | | | | |
| 562 | none | 1/9/29 | 2.62 | 2/15/29 | 3.01 |
| 562B | 1.46 | 1/9/29 | 14.13 | 2/15/29 | 13.42 |
| 563 | none | 1/9/29 | 2.52 | | |
| 563B | 1.46 | 1/14/29 | 13.54 | {1/18/29} | {14.02} |
| | | | | {2/15/29} | {13.21} |
| 695 | none | 1/30/29 | 2.73 | 2/15/29 | 2.56 |
| 695D | 0.51 | 1/30/29 | 6.64 | 2/15/29 | 6.00 |
| 695E | 1.53 | 1/30/29 | 14.01 | 2/15/29 | 13.39 |
| Average of six..... | | | 8.945 | | 8.733 |

It was thought desirable to study the effect of storage or aging upon

¹ Cited by Rogers et al., *Fundamentals of Dairy Science*, p. 71.

² Cornell Univ. Agr. Exp. Sta. Memoir 29, p. 148.

³ Wis. Agr. Exp. Sta. Res. Bull. 57, p. 38 (1923).

the lipid phosphorus content of ice cream mix when preserved with formaldehyde at room temperature and to compare the effect of this treatment with the results obtained when samples were held in the refrigerator unpreserved. Two series of samples of egg-free mix, representing extreme variations in lipid phosphorus content, were prepared. Definite amounts of fresh egg yolk were added to some of the sub-samples. One series was placed in the refrigerator, and another containing formaldehyde (2 drops per ounce) was left at room temperature. Initial analyses of all samples were made for lipid phosphorus content. After 10 days the samples were again analyzed. The data are presented in Table 11, a study of which shows that the lipid phosphorus content was not materially changed by treatment of the samples as described¹.

TABLE 11.

Lipoid phosphorus content and calculated fresh egg yolk content as affected by storage with and without formaldehyde.

| LAB. NO. | DESCRIPTION | INITIAL | | AFTER 10 DAYS | |
|-------------|---|---|---|---|---|
| | | Lipoid phosphorus (mg. per 100 grams) | Calculated yolk (formula) per cent | Lipoid phosphorus (mg. per 100 grams) | Calculated yolk (formula) per cent |
| 2100A | No egg; unpreserved in refrigerator | 4.02 | 0.37 | 4 15 | 0 48 |
| 2100B | No egg; formaldehyde; room temperature | 3 83 | 0 33 | 3 60 | 0 27 |
| 2100C | 2% yolk; unpreserved in refrigerator | 10 95 | 2.16 | 11 56 | 2 32 |
| 2100C | 3% yolk; formaldehyde; room temperature | 15 27 | 3 20 | 14 86 | 3 17 |
| 2082A | No egg; unpreserved in refrigerator | 1 78 | . . | 2 02 | . |
| 2082B | No egg; formaldehyde; room temperature | 1.75 | | 1 70 | . . . |
| 2082C | 3% yolk; unpreserved in refrigerator | 13 14 | 2.73 | 13.19 | 2 74 |
| 2082D | 3% yolk; formaldehyde; room temperature | 12 20 | 2 48 | 12 15 | 2.47 |
| Averages | | 7.868 | 1 409 | 7 904 | 1 431 |

EFFECT OF PASTEURIZATION.

In the same connection the effect of pasteurization of the sample upon the yield of lipid phosphorus was studied in a very limited way. A brief study of Table 12 will show that pasteurization for 40 minutes at 145°F. did not decrease the percentage of lipid phosphorus. In three of the four samples so treated a distinct though small increase in this constituent is to be noted.

¹ Since the samples used in this experiment represent extremes in lipid phosphorus content for commercial ice cream mix, the application of the formula for calculating egg yolk percentage exhibits approximately extreme positive and negative variations (see Table 4).

TABLE 12.

Lipoid phosphorus content of ice cream mix with and without egg yolk as effected by pasteurization for 40 minutes at 145°F.

| LAB. NO. | FRESH EGG YOLK CONTENT | LIPOID PHOSPHORUS (MG. PER 100 GRAMS) | |
|----------|---------------------------|---------------------------------------|----------------------|
| | | Before pasteurization | After pasteurization |
| | <i>per cent</i> | | |
| 695 D | 1.0 | 6.54 | 6.64 |
| 695 E | 3.0 | 13.57 | 14.01 |
| 695 F | 5.0 | 22.94 | 21.74 |
| 695 | none | 2.62 } | 2.73 |
| | | 2.53 } | |
| Averages | | 11.408 | 11.280 |

ANALYSES OF UNKNOWN MIXTURES.

In order to test further the accuracy of the procedure and formulas, two sets of samples of ice cream mix containing varying amounts of fresh egg yolk were prepared. The compositions of the samples were unknown to the writer. The series 695, A, B, and C was prepared with a commercial egg-free mix as a base. Series 848, A, B, and C was prepared with an experimental egg-free mix made up in the laboratory from milk, sweet cream, condensed milk, sugar, gelatin and fresh egg yolk. This mix contained 11.07 per cent fat and 37.0 per cent total solids. The results of analyses appear in Table 13. Very close approximations to the actual egg yolk content were obtained in all cases, except 695 C, which showed a loss of fresh egg yolk of approximately 0.48 per cent. It is possible that this difference may have been due to the faulty mixing of the yolk with the sample. On the average, the calculated percentages of egg yolk were only slightly under the actual amounts present. The average percentage recovery of yolk as fresh egg yolk was found to be 97.63 per cent, and as dry egg yolk 96.06 per cent.

TABLE 13.

Results of examination of eight samples of ice cream mix, to some of which fresh egg yolk was added—composition unknown to analyst.

| LAB. NO. | FRESH EGG YOLK (BY FORMULA) | DRY EGG YOLK (BY FORMULA) | ACTUAL YOLK PRESENT | | DIFFERENCES BETWEEN ACTUAL AND CALCULATED* | |
|----------|-----------------------------------|---------------------------------|------------------------|----------------------|---|----------------------|
| | per cent | per cent | Fresh yolk per cent | Dry yolk per cent | Fresh yolk per cent | Dry yolk per cent |
| 695 | none | none | none | none | | |
| 695A | 4.68 | 2.34 | 4.83 | 2.47 | +0.15 | +0.13 |
| 695B | 1.00 | 0.50 | 1.12 | 0.57 | +0.12 | +0.07 |
| 695C | 7.44 | 3.72 | 7.92 | 4.05 | +0.48 | +0.33 |
| 848 | none | none | none | none | | |
| 848A | 4.02 | 2.01 | 3.83 | 1.93 | -0.19 | -0.08 |
| 848B | 1.16 | 0.58 | 1.14 | 0.58 | -0.02 | 0.0 |
| 848C | 7.46 | 3.73 | 7.55 | 3.81 | +0.09 | +0.08 |
| Averages | 4.293 | 2.147 | 4.398 | 2.235 | +0.105 | +0.088 |

* Differences equal figures to be added to or subtracted from percentages found by formula to equal the actual percentages of egg yolk.

SUMMARY.

The results of a limited study of methods for a quantitative determination of egg solids in ice cream are presented, as well as a method of procedure for the analysis of ice cream to determine the egg yolk content. This method depends upon the quantitative recovery of lipid phosphorus from ice cream by means of the Roesse-Gottlieb (Mojonnier) extraction of the lipoids followed by a colorimetric determination of the phosphorus. Data are presented relative to the lipid phosphorus content of ice cream and various ice cream constituents, also of eggs and egg products¹. By the use of this data, particularly the average values for egg-free ice cream and for dry egg yolk, a formula was prepared by the use of which the percentage of dry egg yolk in ice cream is calculated from the lipid phosphorus content. From this value the fresh egg yolk percentage may be readily approximated by multiplying the former by two. If desired, the results may be expressed in terms of whole egg solids by using in the formula a different constant, which is derived from the average value for lipid phosphorus in whole egg solids. The validity of these formulas has been established and their general application is limited only by the amount of data offered in support of them. A large number of analyses have been made, and in no case has the calculated percentage of egg yolk differed markedly from the amount actually present.

ACKNOWLEDGMENTS.

The writer wishes to express his appreciation of the assistance rendered by Mr. Firman Thompson², Chief of the Division of Chemistry, for advice and encouragement given him during the progress of this work; also to Mr. C. F. Hoyt, in charge of the Dairy Laboratory, for advice and criticism. To Mr. R. W. Newman, bacteriologist of the Dairy Laboratory, the writer is especially indebted for most helpful criticism of the manuscript. He wishes to thank Mr. A. E. Reynolds and Mr. F. W. Milner, ice cream specialists of this department, for procuring samples and furnishing much needed information concerning the history of same. To Dr. W. L. Burdick and to Professor J. H. Norton of the Sacramento Junior College, the writer is indebted for the use of a colorimeter; also to Mr. A. J. Affleck of Affleck Laboratories, for the use of his colorimeter.

¹ Since submitting this manuscript additional work has been done which confirms the data and conclusions presented.

² Deceased, February 2, 1930.

BOOK REVIEWS.

Applied Inorganic Analysis. By DR. W. F. HILLEBRAND, Late Chief Chemist, U. S. Bureau of Standards, and DR. G. E. F. LUNDELL, Chemist, U. S. Bureau of Standards. John Wiley & Sons, Inc., New York, 1929. XIX + 929 pp. 40 figs. 14.5 x 22.75 cm. Price \$8.50.

This book, appearing about four years after the death of the senior author, is largely the work of Dr. Lundell, with the exception of Parts III and IV (206 pp.) in which is given Dr. Hillebrand's well-known treatise on the Analysis of Silicate and Carbonate Rocks, essentially in the form used in Bulletin 700 of the United States Geological Survey (1919). This classic on rock analysis is so well and so favorably known that it will suffice to say that it has been brought thoroughly down to date by attention to the considerable volume of research in this field that has been published since the appearance of Bulletin 700. Needless repetition has been avoided by referring to the general section (Part II) of the present book for the details of many determinations.

Although Parts I, II and V are in the main to be attributed to Dr. Lundell, one must not lose sight of his constant association with Dr. Hillebrand through many years in the laboratories of the United States Bureau of Standards, and throughout the whole book one is conscious of the influence of the Grand Master of Analytical Chemistry in America.

The volume is designated as having special reference to the analysis of metals, minerals and rocks, but in point of fact this limitation means little more than that certain specialized branches of industrial analysis have not been considered. The work is decidedly general and comprehensive in its scope.

Part I (160 pp.) deals with apparatus, reagents and operations. Here is given a very full discussion not only of the apparatus and operations usually treated in the introductory portion of a book on quantitative analysis, but also of precipitations and separations by general group reagents and by special reagents used as precipitants for various elements, once they have been separated from other interfering substances. The specifications and manipulations of weights and balances and of volumetric measuring apparatus are given with great thoroughness, and free use is made of the data and tables of the Bureau of Standards. In a book that is so wide in its scope, and so nearly self-sufficient as is this one, it strikes the reviewer as a pity that the subject of potentiometric titration is not treated a little more in detail. Still, the references to work in this field are very complete.

In Part II (484 pp.) the analytical chemistry of the individual elements is treated in a detailed manner. Again here the thoroughness of the treatment is much in evidence, and it is gratifying to find so full a description of the work dealing with the rarer elements. Sources of error which are commonly overlooked are given special prominence, and one's confidence in the use of the book is heightened by the manifest honesty which pervades its pages. As a single example, in discussing the separation of antimony and arsenic from tin by the classical method of Clarke it is frankly admitted that the authors do not actually know how molybdenum, tellurium, selenium and germanium would behave. They give their guess in the matter, but the operator is thus warned that he had better be on his guard if the presence of these rarer elements is a possibility.

In Part V (38 pp.) a few special analytical methods are given. Also numerous tables show the degree of concordance between results obtained by various analysts in establishing the composition of many of the standard samples issued by the Bureau of Standards.

This book is not intended, of course, to serve as an elementary instruction text in quantitative analysis, but for an advanced course, with proper lecture guidance, it

would serve well. The fundamental theories of analytical chemistry are not treated, as they fall without the scope of a book on applied analysis. As a reference book for the practising analyst, and as a book for further self-study, this volume is an outstanding contribution, and it should find a place in every library, as well as on the shelves of every analytical laboratory. The reviewer feels that any analyst, however thorough his schooling may have been, will get from his reading in this work an awakened understanding of the difficulties to be overcome, and of the limitations of many methods that he has heretofore used with a sense of fancied security.

A semi-conversational style prevails in many pages which suggests that intense interest in the subject, combined with a lack of fear of the editor's scissors, which constitutes one of the charms of British writers, but which is less frequently seen in the works of American scientific authors.—PAUL H. M.-P. BRINTON.

Volumetric Analysis. Volume II, Practical Volumetric Analysis. By I. M. KOLTHOFF. Translated by N. Howell Furman. John Wiley and Sons, Inc., New York. 552 pages, 18 figs. Cloth, 6x9. 1929. Price \$5.00. The chemist will find that the author's promises made in the preface are fulfilled in the text. In the reviewer's opinion the chief value of this treatise is the careful description of the analytical details presented or developed by the author together with the copious list of references to the original work. The author does not claim that the book includes all titration methods that have ever been published; he has limited his field "to those that have an actual practical significance and that have proved to be reliable and free from objection". He has set a difficult standard to attain since "in by far the majority of cases the author considered it mandatory that he himself should test the methods critically and give his judgment".

As an example of the fulfillment of this promise attention is directed to page 78 where the preparation of water for diluting standard alkali is given. The following statement is made: "Most books prescribe boiled distilled water for the preparation of 0.1 *N* alkali. This is correct in principle; yet the carbonic acid content of distilled water that is in equilibrium with the carbon dioxide of the air is so slight that it may be left out of consideration for this purpose". Then after giving the figures which he determined [I. M. Kolthoff, *Biochem. Z.*, 176, 101 (1926)] he does not, like so many others, leave one with no further suggestions, but offers a simple practical method of establishing the equilibrium in 10 hours.

As stated previously, the book contains a vast amount of helpful detail, and limiting factors of the methods presented are given so that one may easily select a method to cover the situation confronting him. Primary standards are particularly stressed, and details of preparing them are given without stint. Indicators are treated fully; a two-page summary of mixed indicators is provided as well as a table of the titration exponent and excess of acid or alkali that is necessary for the indicators that are most commonly used.

Chapter headings give at a glance the wealth of material in this book. Chapter I, Measuring Vessels for Volumetric Analysis. Their Calibration and Testing; II, Practical Principles of Volumetric Analysis; III, Acidimetry and Alkalimetry; IV, Quantitative Neutralizations; V, Displacement Reactions; VI, Hydrolytic Precipitation and Complex-Formation Reactions; VII, Special Methods of Alkalimetry and Acidimetry; VIII, Titrations with Silver Nitrate (Argentometry); IX, Formation of Slightly Dissociated or Complex Compounds. Mercurimetry; X, General Discussion of Indicators for Oxidation and Reduction Process (Oxidimetry); XI, Permanganometry (Oxidimetry); XII, Iodometry. The Properties of the Volumetric Solutions and Their Standardization; XIII, The Practical Methods of Iodometry; XIV, Titrations with Potassium Iodate; XV, Titrations with Potassium Bromate; XVI, Titrations with

Potassium Dichromate; XVII, Ceric Sulfate as a Volumetric Reagent; XVIII, Other Standard Solutions. Titanometry.

The chapter on Ceric Sulfate as a Volumetric Reagent is cited to illustrate the thoroughness with which the author covers the development of a method. In this instance the first worker to use salts of quadrivalent cerium [Lang, *J. prakt. Chem.*, **82**, 129 (1861)] as oxidizing agents in volumetric analysis is quoted, as well as those that followed, up to the date of publication [Willard and Young, *J. Am. Chem. Soc.*, **50**, 1222, 1334, 1368, 1372, 1379 (1928)]. With this new reagent as here prepared, arsenic trioxide, trivalent antimony, iodine, ferrous iron and ferrocyanide may be determined. Nearly all of these methods appeared in 1928 and 1929.

The translator is to be congratulated on the manner in which he has performed his task.—R. B. DEEMER.

NEW BOOKS.

Diatomaceous Earth. By ROBERT CALVERT. The Chemical Catalog Co., Inc., New York City, N. Y. 1930. Price \$5.00. One of the American Chemical Society Monograph Series. In the words of the first paragraph of the preface, "This Monograph is offered as a description of the present day industry of diatomaceous earth, an interpretation of the literature in the light of experience, and an indication of needed discoveries yet to be made in a new and interesting field of research". The titles of the sixteen chapters and subtopics of each attest the accomplishment of the author's ambitions. Readers of *This Journal* will be particularly interested in Chapters VI, "Filtration Medium of Diatomaceous Earth"; VII, Filtration of Sugar Solutions; VIII, Miscellaneous Filtrations with Diatomaceous Earth; and XV, Diatomaceous Earth as Absorbent. Chapter XV considers its use in Disinfectants, Fungicides, Insecticides, Degreasing Wool, and Absorption of Liquid Fertilizers.

The Nature and Properties of Soils. By T. L. LYON and H. O. BUCKMAN. Revised Ed. The Macmillan Co., New York City, N. Y. 1929. Price \$3.50. It is stated on the title page that this is "A College Text of Edaphology", the latter term being indicative of the thoroughly "up to the minute" character of this text. The completeness of the references given bears out the impression of the authoritative and complete modernness of the subject matter. The chapter headings are: I, A Fundamental Conception of the Soil; II, The Supply and Availability of Plant Nutrients in Mineral Soils; III, The Soil Particles and the Physical Nature of Mineral Soils; IV, The Organisms in the Soil; V, The Organic Matter of Mineral Soils; VI, The Methods of Fertility Maintenance for Mineral Soils; VII, The Formation of Mineral Soils and the Origin of Soil Materials; VIII, Classification of Soils and the Soil Survey; IX, The Nature and Utilization of Organic Soils; X, Forms of Soil—Water and Their Significant Physical Relationships; XI, The Plant Relationship of Soil—Water and Methods for Its Control; XII, Water as a Solvent—The Soil Solution and Its Nutrient Losses; XIII, The Soil Reaction, Soil Acidity and Alkalinity; XIV, Liming the Soil; XV, The Nitrogen Economy of Soils; XVI, Fertilizers and Fertilizer Practice; XVII, Green-Manures and Their Management; XVIII, Farm-Manure and Its Utilization.

Corrections.

In Volume 13, No. 1, 1930, on page 39, par. (a), line 7, change "25 grams per liter" to "50 grams per liter". Also on p. 61, par. (a), line 7, change "25 grams per liter" to "50 grams per liter".



BENNETT BATTLE ROSS, 1864-1930.

BENNETT BATTLE ROSS

On April 5 the sad news of the death of Dr. B. B. Ross, Dean of the Department of Science of the Alabama Polytechnic Institute and State Chemist of Alabama, was received in Washington. With Mrs. Ross he had gone to Miami, Florida, for a short vacation, and it was while on this visit that his death unexpectedly occurred. While the many friends of Dr. Ross had known of his poor health during the past year, his sudden end came as a great shock.

His active interest in the work of the Association of Official Agricultural Chemists over a period of more than thirty years resulted in the development of a wide circle of acquaintances and friends in the agricultural experiment stations, State colleges and universities and in the U. S. Department of Agriculture. Indeed, to some of us the annual meetings of the Association of Official Agricultural Chemists will seem lacking and incomplete without Ben Ross, "the tall pine of Alabama", as his many friends in the organization fondly called him.

Dr. Ross was born at Tuskegee, Alabama, December 25, 1864, and was graduated from the Alabama Polytechnic Institute in 1881, later doing graduate work at that institution, and completing his training at the universities of Göttingen and Berlin. He was assistant chemist at the Alabama Polytechnic Institute 1884-1887, and Professor of Chemistry at the Louisiana State University 1887-1893; in '93 he returned to the former as Professor of Chemistry. From 1908 to 1921 he was Dean of the College of Agricultural Sciences, and, from 1921 until his death, Dean of the Department of Science in that institution. During the years 1919-1920 he was acting president of the institution. He had been State Chemist of Alabama since 1893. He was a Fellow of the A. A. A. S., member of the American Chemical Society, and past chairman of the Alabama section. He served for many years on Subcommittee A of the Association of Official Agricultural Chemists, and was President of the association for the year 1896. He was also past president of the International Association of Food and Dairy Chemists. He was a member of Alpha Tau Omega.

In Dr. Ross was combined to a remarkable degree the genius and ability of both the teacher and the investigator, that rare combination which means so much to the student who may have the good fortune to come under the influence of such a leader. For thirty-seven years he served the Alabama Polytechnic Institute as Dean and Professor of Chemistry. He made important contributions to the advancement of science and was the author of numerous scientific papers and books, one to be published. Only recently he and his collaborators had completed a chapter on potash as a fertilizer for the revision of volume two of Principles and Practice of Agricultural Analysis, soon to be issued by the association. As a teacher he has left a rich heritage to the state and to the nation in the many students he has so ably trained and in whom he has not only inspired an enthusiasm and a love of science but has also inspired the will to do right, to acquire culture, to render service, to be Christian gentlemen.

Dr. Ross was a man of commanding presence, with a striking air of intellectual vigor which marked him in any audience in which he happened to be. A droll sense of humor and an engaging personality readily attracted acquaintances who became, and invariably remained, his friends. As one who has served with him on numerous committees and has seen him in action under numerous trying circumstances, I can pay him no higher tribute than to say that I have never known him to lose his poise or good humor, and in thirty-five years of close association have never known him to give voice to an unkind thought.

The last years of his life were given unreservedly to the upbuilding of his State and the Alabama Polytechnic Institute. Largely through his efforts there has recently been dedicated on the campus of that institution a new, splendidly equipped chemical laboratory, which will stand in the years to come as a monument to his devotion to his Alma Mater. The tremendous energy which he was putting into the consummation of this great work was recognized at the time by his many friends, who fully appreciated the serious drain upon his vitality, but no one ever thought of suggesting to him the idea of his doing less, even though it meant the supreme sacrifice. The loss of such a man as Dr. Ross is irreparable to his friends and associates. He was one of those stalwart characters who, in some way, bridged the gap between the vigorous, awakened spirit of a new South and the gracious, cultured, hospitable ante-bellum period. One is privileged to know but few such men in a lifetime.

W. W. SKINNER.

EDITORIALS.

NATIONAL SOYBEAN OIL MANUFACTURERS' ASSOCIATION.

During the last few years the acreage of soybeans grown for seed purposes has increased markedly in the middle western states. A number of soybean crushing mills have been established for pressing the oil from the beans, and present conditions indicate rapid expansion in production. This increasing importance of the soybean crop in the United States was emphasized by the organization of The National Soybean Oil Manufacturers' Association in Chicago, May 21, 1930.

The object of the association is to promote the welfare of the soybean oil industry by exchange of information among its members, to prevent attendant evils and trade customs contrary to sound business principles and to formulate constructive policies for the cooperation of its members as a means of eliminating grievances by fair and equitable adjustment.

Membership is made up of individuals, firms or corporations engaged in manufacturing or refining soybean oil. Provision is also made for associate membership by individuals, firms or corporations not engaged in manufacturing or refining soybean oil and for special associate membership of individuals who have rendered meritorious service in the soybean industry.

The constitution of the association provides for a number of committees, among which is the Committee on Research and Trade Promotion, the functions of which are to study the market for soybean products with a view to expansion through research and trade promotion, to encourage research by colleges and universities and to cooperate with all agencies engaged in research for the benefit of the industry.

The association adopted a detailed set of trading rules and a set of specifications for purity and quality of crude domestic raw soybean oil. Methods of analysis are those of the American Society for Testing Materials given under "Tentative Specifications for Soybean Oil", as outlined in the Proceedings of the A. S. T. M. Specification D 124-22T, pp. 748-752, volume 22, except that the iodine number shall be determined by the Wj's method as outlined in *Industrial and Engineering Chemistry*, 18, 1346 (1926).

FORMS OF NITROGEN IN PLANTS.

The accurate quantitative determination of the commonly occurring water-soluble nitrogenous substances is difficult even in the usual plant extracts. In special cases like the tobacco plant, which contains nicotine, and other plants containing cyanophoric glucosides the usual methods are not satisfactory.

Vickery and Pucher¹ have developed an improved and satisfactory method for the determination of ammonia and amide nitrogen in tobacco extracts. It is based on the principle that nicotine is absorbed by permutit only to a limited extent and gives no color with Nessler's reagent, while ammonia can be removed quantitatively from a faintly acid solution of permutit and subsequently set free and determined. These workers point out that the method may be useful in the investigation of other plants since usually the total volatile base is titrated and reported as ammonia while there is no assurance that other volatile bases are not present and included in the ammonia.

Robinson² describes methods for the estimation of various forms of nitrogen in cyanophoric plants. He found it advisable to remove tannins by precipitation with colloidal

¹ *J. Biol. Chem.*, 83, 1 (1929); Conn. Agr. Expt. Sta. Bull. 311, p. 234, 1930.

² *Biochem. J.*, 28, 1099 (1929).

ferric hydroxide or alkaline lead acetate, because otherwise irregular results were obtained in the amino nitrogen determination. The usual method of hydrolysis in the determination of amide nitrogen cannot be used in the presence of cyanophoric plants owing to the partial decomposition of cyanide to ammonia under such conditions. In order to avoid this interference the amide nitrogen is hydrolyzed with tartaric acid.

These papers serve to emphasize the limits of application of the usual methods and the need for more extended study of these methods.

FIRST DAY.
MONDAY—AFTERNOON SESSION—Continued.

DRUG SECTION.

REPORT ON DRUGS.

By ARTHUR E. PAUL (U. S. Food, Drug and Insecticide Administration,
Chicago, Ill.), *Referee.*

For a number of years this association has carried on extensive work on the subject of drugs. The field is so broad that the problem of selecting the most important subjects gives the referee some concern. The selection is usually made so as to include the most potent products which are used by physicians in emergencies and in the treatment of the more serious ailments, provided, of course, that methods are not available in the U. S. Pharmacopeia or in the National Formulary. Consideration is invariably given also to the matter of making the respective determinations in mixtures that may contain interfering substances, with which feature the U. S. P. and National Formulary are not particularly concerned.

It is the policy to avoid any duplication of the work of the U. S. Pharmacopeia; in several instances the study of subjects was discontinued when it was learned that methods would be included in the new revision of that work. On the other hand, modifications of the U. S. P. methods have been studied, or new methods, applicable in the examination of mixtures, have been devised.

Since the most important subjects have now been studied, or are being studied, attention can be directed to some of the less important items, as well as to new products which are constantly appearing on the markets.

Rather recently there has been established in the Drug Control office of the U. S. Food, Drug and Insecticide Administration a special investigative unit for the purpose of compiling drug methods. This unit has an object similar to that of this association—the endorsement of suitable drug methods; it is taking up products in the order of the frequency with which they are prescribed by physicians. The list may be useful to next year's referee in deciding upon suitable new subjects for study and adoption by this association or by the U. S. Pharmacopeia.

At the present time a combined committee of the American Drug Manufacturers' Association and the American Pharmaceutical Manufacturers' Association is also compiling a set of drug methods. These, however, are intended to serve the special purpose of manufacturers

who have positive knowledge of the ingredients in each sample examined and use the results for their factory control. In many instances their methods differ from the methods of this association only in details that are permissible because of the intended purpose. In some cases, however, new principles may be involved in connection with substances for which methods have not yet been studied by this association, but which may be helpful and suggestive to next year's referee.

During the present year twenty associate referees were assigned to individual subjects. Of these, eighteen submitted reports; two were unable, owing to unforeseen contingencies, to carry on sufficient work to warrant the submission of a formal report. Although it was not possible to close a number of the topics, it is felt that much advance was made in the status of the association's drug work.

It is recommended—

(1) That methods for the determination of the following substances be adopted as tentative:

Mercuric Iodide
 Brucine
 Caffeine
 Ephedra Assay
 Salicylates and other phenols

(2) That the following subjects be considered closed:

Fluidextract of Ginger
 Phenols and other Salicylates

(3) That further study be given to the respective methods for—

| | |
|-----------------------------------|---------------------|
| Crude Drugs | Menthol |
| Radioactivity in Drugs and Waters | Iodides in Mixtures |
| Laxatives and Bitter Principles | Ephedrine |
| Mercurials | Chenopodium |
| Microchemical-Alkaloids Methods | Bismuth Compounds |
| Santonin | Vitamins |
| Ether | Phenolsulfonates |
| Bioassay of Drugs | Thymol |

(4) That the following subjects, on which no reports were rendered, be continued:

Terpin Hydrate
 Bromides and Chlorides

(5) That the following new topics be studied:

| | |
|----------------------|-----------------------------------|
| Sulfonal and Trional | Guaicol Carbonate in tablets |
| Calcium Lactate | Sulfur Ointment and Zinc Ointment |
| Emetine | |
| Camphor Liniment | |

(6) That the subject of chloroform and carbon tetrachloride be reopened.

(7) That the tentative method for the determination of atropine be slightly amended.

(8) That the standards proposed by the associate referee for pituitary powders be referred to the U. S. P. Revision Committee.

(9) That the incoming referee give careful consideration to the work of the Research Unit, Drug Control, Food, Drug and Insecticide Administration, and of the Combined Contact Committees of the American Drug Manufacturers' Association and American Pharmaceutical Manufacturers' Association, for the purpose of selecting from their lists any topics which may appear sufficiently important and desirable to warrant study by this association.

(10) That the following comments on reports submitted by associate referees be given consideration.

CRUDE DRUGS.

The associate referee calls attention to an unofficial variety of *viburnum*, which grows in the southern states along the Atlantic coast, and gives a complete description of the plant as well as of the drug. The information is interesting, but it is not, and probably cannot be, presented in such form as to constitute a method suitable for adoption by the association. Since *viburnum* is not a particularly important drug, it is not deemed desirable to devote further attention to the subject. It is believed that consideration of histological methods in this association should be confined to the more important crude drugs.

Since various species of aconite, which are not the U. S. P. variety, have been offered for entry, it is recommended that this drug be studied with a view to formulating concise directions for distinguishing the official from allied species.

RADIOACTIVITY IN DRUGS AND WATER.

The progress made by the associate referee on this difficult and extremely technical subject is very satisfactory. Some collaborative results are included in his report, but it is considered necessary to obtain further results. The associate referee has made arrangements for such additional investigation, and his recommendation for further study should be approved.

LAXATIVES AND BITTER TONICS.

Last year the associate referee propounded the theory that a separation of anthraquinones into two classes, "free" and "combined", would make possible a distinction between the inactive and active components.

A method for making the separation was devised and submitted to collaborators. The results were encouraging, but the method requires additional study.

During the present year the associate referee revised his method. He also established contact with pharmacologists in the Food, Drug and Insecticide Administration in Washington for the performance of the necessary investigation to determine its value, but it was found impossible for them to devote the necessary time to the matter. Since this is an important subject, it is recommended that this study be continued along the lines planned by the associate referee.

MERCURIALS.

The U. S. P. includes an electrolytic method for the assay of mercuric iodide that is entirely satisfactory. However, since some laboratories are not equipped to carry on this determination, it seems desirable to have available an approved chemical method. The associate referee studied three methods and recommends tentative adoption of the iodate method, which is simple, rapid and accurate, as shown by the collaborators' results. This recommendation is approved, as is also his further recommendation that next year methods be studied for the examination of calomel and mercuric oxide ointments.

MICROCHEMICAL METHODS FOR ALKALOIDS.

Consistent progress on this subject has been made during the past four years, fourteen alkaloids having been studied. Of these, one, heroine, is now official and appears in the association's book of methods; four, cocaine, codeine, morphine and strychnine, were made tentative in 1927; six, namely, atropine, pilocarpine, quinine, quinidine, cinchonine, and cinchonidine, were made tentative in 1928; and in accord with the associate referee's recommendation, two additional alkaloids, brucine and caffeine, are now presented for similar action.

The associate referee and his collaborators also worked on methods for the determination of ephedrine. Owing to the importance recently attached to this alkaloid, the desirability of having satisfactory methods for its identification is evident. The recommendations of the associate referee are approved.

TERPIN HYDRATE.

The associate referee was unable to make a report on this subject. It is recommended that the promising method devised last year be studied collaboratively.

SANTONIN.

The associate referee made sufficient progress on this very difficult subject to recommend certain methods for tentative or official adoption. Since they have not been subjected to collaborative study, however, it is recommended that they be studied next year, possibly in conjunction with other procedures that may be available.

ETHER.

Somogyi's oxidation method, which is the only one that has been studied, warrants further study. It was studied by G. C. Spencer, Associate Referee, in 1927, but the work was difficult and it was not completed. No report was made in 1928, but during the present year a large amount of systematic work was done to simplify it and to make it applicable to mixtures. It is recommended that this work be continued during the coming year.

BIOASSAY OF DRUGS.

The U. S. P. (X) includes "pituitarium" and "liquor pituitarii". For the former no assay or standard is given, but a biological assay is given for the latter. This assay method is based on a standard powdered pituitary, for the preparation of which directions are given. Last year, in a contributed paper, McClosky and Munch reported on their work and recommended a standard for the official posterior pituitary powder and also a standard for desiccated whole pituitary powder. During the present year some additional work was performed by the associate referee, and based upon this he recommends that the U. S. P. assay for liquor pituitarii be adopted by the A. O. A. C., as well as the standards mentioned above.

The adoption of standards for drug preparations is hardly a function of this association and it is not the policy to adopt methods which have been adopted by the U. S. P. It is believed, however, that the work done is of considerable value and that it would be of interest to the Revision Committee of the U. S. P. It is therefore recommended that this report be referred to that committee for consideration in connection with the 11th revision of the Pharmacopeia.

It is recommended that no further work be done on the subject of pituitarium, but that the subject of bioassay of drugs be continued with the study of biological products for which no method has been adopted by either this association, the U. S. P., or the National Formulary.

FLUIDEXTRACT OF GINGER.

No report was made by the associate referee, but in view of the similarity between this subject and that of essence of ginger and of the

fact that the product is not especially important, it is not considered advisable to continue this topic.

EPHEDRA.

The associate referee's recommendations for tentative adoption of the assay method for ephedra and further study of his method for ephedrine in mixtures are approved.

THYMOL.

Last year a method for the determination of thymol was tentatively adopted by this association. The topic was continued, however, for the purpose of devising details for this determination in complex mixtures, particularly in the presence of eucalyptol, methyl salicylate and menthol. Such details were worked out by the associate referee and sufficient work was performed by him and one of his collaborators to show that the method has merit. Nevertheless, further collaborative study should be made, and it is therefore recommended that the subject be continued.

MENTHOL.

Last year the associate referee submitted a method on which several collaborators reported slightly high results. During the present year he made a study to ascertain the causes for these slight discrepancies, but he was unable to arrive at a conclusion. However, the collaborative results as a whole are so acceptable that it would seem to be satisfactory for the associate referee to re-submit his method to collaborators, with any modifications which he may deem advantageous.

BROMIDES AND CHLORIDES.

Last year the associate referee submitted the results of his study of Winkler's method, but he was not able during the year to complete his work on the subject and to carry on collaborative study. Last year's recommendations are therefore repeated¹.

OIL OF CHENOPODIUM.

The associate referee and his co-worker, G. S. Weiland, made a very exhaustive study of the Paget and Nelson methods for the determination of ascaridole, which is stated to be the therapeutically active constituent of oil of chenopodium. He shows the greater accuracy and dependability of the Paget method, and the results obtained leave little to be desired in point of accuracy. The subject has been materially advanced through this year's work, and it now remains to study the method collaboratively, present the method in the general form used

¹ *This Journal*, 12, 76, 302 (1929).

by the association, and to extend the method to the examination of mixtures and possibly to the assay of the crude drug.

BOTH SALICYLATES AND PHENOLS IN MIXTURES.

In view of the satisfactory results reported by the associate referee, his recommendation for tentative adoption is approved.

SMALL QUANTITIES OF IODIDES IN MIXTURES.

Two methods were studied; both were found satisfactory for inorganic mixtures but not so in the presence of certain organic substances. It will therefore be necessary to continue the subject for another year in order to study this feature.

BISMUTH COMPOUNDS.

The associate referee on this new topic confined his investigation to the collaborative study of a method for the determination of inorganic bismuth compounds in tablets. The method seems promising, but the results were not deemed by him to be sufficiently concordant to warrant adoption of the method. His recommendation for further study is therefore approved.

COLORIMETRIC METHODS FOR VITAMINS.

Examination of foods and drugs for vitamin activity is at present carried on by biological methods. However, extensive work has been done to study chemical means of identification and possibly estimation. Because of the extreme importance of this subject, and the desirability of having chemical tests and methods, it was assigned for study during the present year. Accordingly, a thorough search of the literature was made under the direction of the associate referee. Published methods were abstracted, and, together with a complete bibliography, they are submitted to the association as a contributed paper by E. M. Bailey, the associate referee, in collaboration with H. J. Fisher. The information contained therein should be of great value next year in continuing this subject.

PHENOLSULFONATES.

Because the U. S. P. assays for phenolsulfonates are not entirely satisfactory, the associate referee devised a method which appears to be sound in principle. Results obtained by him are promising, but unfortunately he received reports on samples submitted from only two collaborators, and they are not so close as might be desired. Further study of the method is necessary. Since the samples submitted were in the form of powdered mixtures, and there is necessarily a question as

to their absolute uniformity, as well as to their purity, it is suggested that in next year's work these features be given consideration.

ATROPINE.

The tentative method for the determination of atropine in tablets is not entirely satisfactory in practice owing to hydrolysis of the free alkaloid during the evaporation of the last portion of the chloroform solution. The matter was given exhaustive study by Watkins and Palkin, and a report of their findings was published¹.

This work will justify a slight change in the present tentative method, which change renders the procedure accurate and satisfactory. It is therefore recommended that the last part of the tentative method be changed to read as follows:

Evaporate on a steam bath to about 5 cc. Add a measured excessive volume of 0.02 *N* sulfuric acid and continue the evaporation until the odor of chloroform has disappeared. Cool the solution and titrate back with 0.02 *N* sodium hydroxide, using 1 drop of methyl red indicator. 1 cc. of 0.02 *N* acid = 5.784 mg. of atropine or 6.945 mg. of atropine sulfate.

CHLOROFORM AND CARBON TETRACHLORIDE.

Last year a method for the determination of chloroform and carbon tetrachloride was adopted; it involves saponification direct, that is, without previous distillation. Since, however, the method involves the determination of total silver precipitate before and after saponification, it would hardly seem satisfactorily applicable to mixtures containing large amounts of chlorides. These will frequently be encountered, especially in connection with cough sirups, which often contain large proportions of ammonium chlorides, and at times also ammonium iodide. In such instances it seems quite imperative that the chloroform be separated from the halides.

An article by Roberts and Murray² records satisfactory results by the addition of sufficient alcohol to permit of more ready distillation and at a lower temperature. This seems to be a very desirable suggestion, and it is therefore recommended that an associate referee be appointed to give consideration to this method in conjunction with the present tentative method, in order to decide whether any further amendment in the latter should be made.

It was moved, seconded and adopted that the association endorse the name of Dr. Lyman Spalding, the father of the U. S. Pharmacopeia, for the Hall of Fame, New York University.

¹ *J. Am. Pharm. Assoc.*, 16, 1044 (1927).

² *Am. J. Pharm.*, 101, 654 (1929).

REPORT ON CRUDE DRUGS.

THE ANATOMY AND IDENTIFICATION OF KENTUCKY BLACK HAW BARK.

By HEBER W. YOUNGKEN¹ (Massachusetts College of Pharmacy,
Boston, Mass.), *Associate Referee*.

In 1927 the associate referee received from a well-known crude drug dealer samples of root and stem barks purported to have been gathered in Kentucky, which were labeled, respectively, "Kentucky Black Haw Root Bark" and "Kentucky Black Haw Tree Bark". Leaf and fruiting branches, as well as stems and roots of the trees from which the barks were gathered, were also sent for identification. A comparison of this material with materials gathered by the writer from living plants growing in the Arnold Arboretum of Harvard University in the summers of 1928 and 1929 and with fresh specimens collected in Clinton County, Kentucky, in June, 1929, led to the conclusion that the source of the bark was *Viburnum rufidulum* Raf. or Southern black haw.

A description of the plant as well as the gross and microscopic features of the root and stem barks of Kentucky black haw are hereby presented.

DESCRIPTION OF *VIBURNUM RUFIDULUM* RAF.

Viburnum rufidulum Raf. or Southern black haw is a large shrub or small tree attaining a height of about 30 feet, with stout, rigid branches having obtuse, rusty-hairy winter buds and elliptic to obovate, usually obtuse and serrulate leaves, 2 to 4 inches long, which are glabrous and shining and dark green on the upper surface, rusty pubescent on the veins beneath, especially toward the base, and usually with narrowly winged, rusty pubescent petioles up to $\frac{1}{2}$ inch in length. The flowers are white and arranged in sessile cymes. The fruits are dark blue, ellipsoidal, glaucous drupes with a flat stone. Rehder and Bailey both report its distribution from Virginia to Florida, west to Illinois and Texas. This plant is hereby identified as the source of commercial Kentucky black haw bark, which has been on the drug market for some time.

THE ROOT BARK.

The root bark occurs in irregular or transversely curved pieces or quills from 1.5 to 10 cm. in length and from 0.5 to 3.5 mm. in thickness; outer surface varying from grayish-brown, light brown, yellowish-brown to brown, irregularly longitudinally striated to wrinkled and furrowed, occasionally transversely fissured, and showing cork patches on old bark, reddish-brown to purplish-brown to purplish where cork is abraded; inner surface pale yellowish and frequently marked with irregular blotches

¹ Presented by L. E. Warren.

or streaks of reddish-brown, longitudinally striated; fracture brittle and uneven, exhibiting a brown cork, a reddish-brown cortex and whitish to reddish-brown inner bark containing yellowish groups of stone cells; odor valeric acid like, becoming very strongly so when treated with sirupy phosphoric acid; taste bitter and astringent. Upon treating the inner surface of this bark with a 1 : 100 solution of freshly prepared ferrous sulfate, a greenish-black color is evident.

HISTOLOGY OF ROOT BARK.

The cork is up to 40 or more layers of cells in width, which are tangentially elongated in cross sections. The largest of these cells are up to $154.8\ \mu$ long and $72\ \mu$ in radial diameter. The phellogen is composed of a layer or more of tangentially elongated, thin walled, somewhat compressed cells.

The secondary cortex varies in diameter, according to age. It is composed of pitted parenchyma cells which are tangentially elongated in cross section. Many of these contain rosette crystals of calcium oxalate, while a number of them contain either starch, brownish-red amorphous masses or tannin. Imbedded in this region are numerous groups of stone cells and scattered stone cells. The groups are either tangentially elongated or irregularly circular to oval in outline with irregularly indented margins as viewed in transverse section. An outstanding feature of the majority of the groups is their deeply indented irregular outline. The larger groups showed as many as 80 stone cells in cross section, the groups measuring up to $1020\ \mu \times 510\ \mu$ in these sections. The individual stone cells are of a large variety of shapes and show varying degrees of lignification and pore canal branching. In radial longitudinal sections and in preparations made by Schulze's method they were up to $680\ \mu$ in length.

The phloem varies in breadth but is averagely broader than the cortex. It is composed of phloem parenchyma containing either rosette aggregates or monoclinic prisms of calcium oxalate, starch grains, tannin, or brown amorphous masses. Through the outer and middle portions of the phloem are numerous stone cell groups and isolated stone cells, which resemble those of the cortex. Coursing outward through the phloem in straight or curved fashion are many medullary rays which tend to converge in groups. Some of these are interrupted by the stone cell groups, while others bend around them. The medullary rays observed in tangential sections are from 1-3 cells in width, the majority being 1 cell wide, fewer 1-2 cells wide, fewest 1-3 cells wide. The cell contents resemble those of the parenchyma of the cortex and phloem.

Crystal fibers containing rosette aggregates of calcium oxalate were abundant in the cortex and phloem. The rosette aggregate crystals

were up to $54\ \mu$ in diameter, while the monoclinic prisms studied ranged up to $32.4\ \mu$ in length.

The starch grains were simple spheroidal to oval, the latter often with a beaked end. Most of them were up to $4\ \mu$, a few up to $8\ \mu$, fewer up to $15\ \mu$.

THE STEM BARK.

The stem bark occurs in irregular, transversely curved or quilled pieces 1.5 cm. to 15 cm. in length and up to 6 mm. in thickness. Outer surface is silvery gray on young bark with raised circular lenticels, grayish-brown to blackish on older bark or reddish-brown where cork has scaled off. The old bark shows thick cork with numerous irregular longitudinal, oblique and transverse fissures; inner surface pale yellowish to reddish-brown or yellowish with reddish-brown blotches and streaks, longitudinally striated; fracture short and uneven, fractured surface showing a grayish to blackish outer bark, a greenish-brown to reddish-brown middle bark and a light brown to whitish inner bark in which dull yellow stone cell groups are visible under a hand lens. The odor is faintly like valeric acid, becoming only slightly more pronounced when treated with sirupy phosphoric acid. The taste is astringent and bitter. The inner surface when treated with a 1 : 100 solution of ferrous sulfate is colored greenish-black.

HISTOLOGY OF STEM BARK.

The cork zone is of variable width and composed of more or less isodiametric to tangentially elongated cells in cross sections, polygonal to rounded polygonal in surface sections with suberised and lignified walls. The older cork cells are compressed and for the greater part tangentially elongated, with brownish contents. There is a tendency of the outer and inner walls to be thicker than the radial walls, but this is by no means constant. Frequently islets of stone cells are imbedded within the old cork or between it and the young cork. The young cork appears clear and open looking and is composed of radially and tangentially elongated cells with walls often wavy. The cork cells were up to $72\ \mu$ long and $50.4\ \mu$ wide.

The phellogen is composed of tangentially elongated meristematic cells.

The cortex and pericycle contain tangentially elongated parenchyma with starch, tannin, fixed oil and brownish amorphous contents. Scattered through these regions are many stone cell groups and isolated stone cells. As the stem grows older, secondary cork cambia originate in the depths of the cortex and pericycle and outer phloem, forming in these zones wavy cork layers that contain groups of stone cells. In old stem bark sluffing of the outer layers occurs as far as the last secondary

cork cambium and cork formed in the outer phloem, so that in the real old bark the medullary rays reach outward to the cork cambium. While most of the medullary rays are 1-2 cells in width, there are quite a few 1-3 cells wide.

The phloem is a relatively broad zone consisting of sieve tubes and phloem parenchyma, the latter containing either starch, tannin, brownish amorphous contents (masses) or rosette aggregates or monoclinic prisms of calcium oxalate. The medullary rays course through this region in straight to slightly curved or wavy fashion and contain elements similar in character to those found in the phloem parenchyma. Some of them are interrupted by groups of stone cells, which along with isolated stone cells are abundant in the outer and middle phloem regions. The stone cell groups appear close together in the phloem, cortex and old cork. They are averagely smaller than in the root bark of this species. In radial longitudinal sections one stone cell group measured $850\ \mu \times 510\ \mu$. Many of the individual stone cells observed in preparations made by Schulze's process are sinuate-toothed along the margin. They show a large variety of shapes and sizes.

The margins of the stone cell groups are irregularly rounded, oval or oblong, crenate to toothed and indented. Numerous crystal fibers containing rosette aggregates and a number containing monoclinic prisms as well as rosette aggregates and monoclinic prisms of calcium oxalate occurred in cortex, pericycle and phloem. The rosette aggregates were up to $54\ \mu$ while the monoclinic prisms were up to $21.6\ \mu$.

The starch grains are mostly spheroidal and up to $4\ \mu$, a few are ovoid and beaked and up to $8\ \mu$ in length.

Numerous droplets of a fixed oil separated when sections of the bark were placed in chloral hydrate solution.

The tannin cells took on a greenish coloration when sections were examined in 1 : 1000 solution of ferrous sulfate.

RECOMMENDATION¹.

It is recommended that the subjects of ephedra and aconite be given especial attention in next year's report.

REPORT ON RADIOACTIVITY IN DRUGS AND WATER.

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.),
Associate Referee.

During the first quarter of 1929, three synthetic samples containing known amounts of radium were submitted to F. E. Brown, of Iowa State College, Ames, Iowa; to Herman Schlundt, University of Missouri,

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 63 (1930).

Columbia, Missouri, and to C. H. Badger of this Administration. In September, Brown advised that the apparatus necessary for making the examinations had been purchased and installed, but that the work could not be completed until later. In October, Schlundt reported results obtained by one of his graduate students, Ralph K. Fulton, on sample No. 1, which consisted of Washington City tap water mixed with a definite quantity of standard radium solution. Badger reported results on all three samples.

It seems desirable to include in this year's report only the results obtained by Fulton and Badger on the sample of water, and to reserve Badger's results on the samples of salt and Kaolin until next year, when the results of the other collaborators will be available.

The radioactive water sample was prepared as follows: 25 cc. of a standard radium solution, prepared by the U. S. Bureau of Standards on May 11, 1915, was diluted to 1000 cc. with 1 + 1 hydrochloric acid at 20°C.; 100 cc. of this solution was diluted to 1001.8 cc. at 22°C. with Washington, D. C., tap water and 100 cc. portions of this second solution were taken for analysis. Each cubic centimeter of the standard radium solution, prepared by the U. S. Bureau of Standards, contained, when prepared in 1915, 12.3 millimicrograms (12.3×10^{-9} grams) of radium which had decreased through disintegration to 12.234 millimicrograms by December, 1928. The quantity of radium contained in each 100 cc. portion taken for analysis was therefore

$$\frac{12.234 \times 25}{10 \times 10.02} = 3.05 \text{ millimicrograms.}$$

The following directions for analyzing the sample were sent to the collaborators:

Radium water sample No. 1.—Pipet 100 cc. of the sample at 20°C. into a 300 cc. florence flask. Add about 15 cc. of concentrated hydrochloric acid and dilute with distilled water to about 200 cc. Boil 20 minutes and dilute with boiled distilled water to about 250 cc. Allow to cool somewhat; seal while still warm. Note time of sealing. From this point proceed as described in the report on radioactivity in drugs and water for October, 1924, for the examination of clear solutions¹.

The results reported by the collaborators are given in Table 1.

TABLE 1.
Radium in water sample.*

| COLLABORATOR | FOUND | PRESENT | DIFFERENCE |
|-----------------|-------|---------|------------|
| Ralph K. Fulton | 3 16 | 3 05 | +0 11 |
| | 3 17 | " | +0 12 |
| | 3 18 | " | +0 14 |
| Cecil H. Badger | 2 99 | " | -0 06 |

* Results expressed in billionths of a gram, i. e., in millimicrograms.

¹ *This Journal*, 8, 531 (1925).

The results submitted by Fulton represent the first independent check of the method that it has been possible to obtain, Badger having assisted in perfecting the procedure. The results obtained by the collaborators are considered to be satisfactory, but further collaborative work is essential.

RECOMMENDATIONS¹.

It is recommended that the methods previously submitted for the preparation of samples and the determination of radioactivity² be further tested by collaborative work during the coming year.

REPORT ON LAXATIVES AND BITTER TONICS.

By ELGAR O. EATON³ (Food, Drug and Insecticide Administration, San Francisco, Calif.), *Associate Referee*.

This year's method is a refinement of the one proposed last year. More detail as to the exact manipulations is given in an attempt to standardize the procedure and to overcome objections noted by collaborators.

APPARATUS.

*Type C Continuous Extraction (Fig. 1).*⁴

REAGENTS.

- (a) *Normal sodium hydroxide solution*.—Saturated with salt.
- (b) *Hydrochloric acid solution* (1 + 1).
- (c) *Sulfuric acid solution* (1 + 1).
- (d) *Acetic acid solution* (1 + 100).

METHOD.

(The entire procedure up to the drying of the final extractives should be completed within a working day, to prevent possible chemical changes not yet understood.)

Introduce chloroform into the continuous extraction apparatus to within 2 inches of overflow. Adjust a 200 cc. Erlenmeyer flask, carrying about 125 cc. of chloroform, to apparatus, with a tight-fitting tin-foiled cork. Into the inner tube of the apparatus introduce 5 cc. of the recently shaken cascara preparation by means of a well-drained pipet. Add 20 cc. of water and 1 cc. of reagent d to the cascara layer. Connect the apparatus to a condenser with a well-fitting tin-foiled cork.

Adjust the burner and reflux rapidly (using an asbestos ring to prevent over-heating) for about 3 hours. At this time the chloroform in the tube will be colorless. Disconnect the flask (do not discard contents of apparatus—see further directions). Transfer the chloroform to a separatory funnel at once and shake out while still hot, with 20 cc. of reagent a, releasing pressure from inverted funnel by means of stopcock. Repeat

¹ For report of Subcommittee B and action of the association, see *This Journal*, 12, 64 (1930).

² *This Journal*, 8, 531 (1925); 10, 362 (1927).

³ Presented by L. E. Warren.

⁴ *J. Ind. Eng. Chem.*, 17, 612 (1925).

the extractions with reagent **a** to exhaustion (about 4 shakeouts is all that is necessary, if the analyst has worked with the hot liquid).

Combine the alkaline aqueous solutions and wash with 25 cc. of chloroform. Discard the chloroform, but do not discard any insoluble residue that is salted out. (A tendency to emulsify is overcome by agitating the chloroform layer by means of a wire.)

Add reagent **b** to the alkaline solution to a decided acidity, indicated by a change of color to bright yellow. Extract at once with 25 cc. of chloroform, repeating extraction to exhaustion; about six 25 cc. portions are necessary. Combine chloroform and wash with 5 cc. of water. Run the chloroform through a plug of cotton wetted with chloroform. Wash the water with chloroform and transfer the combined chloroform to a tared beaker. Evaporate, and dry at about 98°C. for 3 hours. Cool and weigh. Weight multiplied by 20 equals grams per 100 cc. of free and salt-occurring hydroxyanthraquinones, probably inactive, but it helps to show the original activity of the drug.

Recharge the Erlenmeyer flask with 125 cc. of chloroform and connect to apparatus, which still carries the chloroform exhausted acetic acid solution of original sample. Add 10 cc. of reagent **c** to the cascara layer by means of a pipet.

Connect to the condenser, and proceed exactly as outlined above, beginning with line headed "Adjust burner * * *". Weight multiplied by 20 equals grams per 100 cc. of oxyanthraquinones, separated by sulfuric acid digestion.

This last extraction should represent an index of the drug's activity; that is, the glucosides and resinous bodies which were physiologically active in the original preparation have been hydrolyzed and an end product which is not necessarily a laxative is determined.

It is recommended that this work be continued¹.

REPORT ON MERCURIALS.

By R. S. ROE² (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

In compliance with the plan for work on mercurials this year, attention was given to methods for the assay of mercuric iodide tablets, calomel ointments and mercuric oxide ointments.

MERCURIC IODIDE TABLETS.

Of the methods appearing in the literature, the following three seemed to offer the best possibilities, and they were studied for the assay of mercuric iodide tablets.

- (I) Rupp formaldehyde reduction, and subsequent iodometric titration.
- (II) Sulfide method of Bender³.
- (III) Potassium iodate method⁴.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 64 (1930).

² Presented by H. Runkel.

³ *J. Ind. Eng. Chem.*, 6, 753 (1914).

⁴ Jamieson, Volumetric Iodate Methods. The Chemical Catalog Co., Inc.

The Rupp formaldehyde reduction procedure consists in the treatment of a weighed portion of the sample in a 300 cc. glass-stoppered flask with 25 cc. of water and 2.5 grams of potassium iodide. This is followed by the addition of 30 cc. of 4 per cent sodium hydroxide, 5 cc. of 5 per cent gum acacia solution, and 3 cc. of formaldehyde. After 20-30 minutes, the mixture is acidified with acetic acid, and an excess of standard iodine is added. After the solution and oxidation of the reduced mercury are complete (about $1\frac{1}{2}$ hours), the excess iodine is titrated with standard sodium thiosulfate.

As modified by Spencer¹, the formaldehyde is omitted when sufficient lactose is present to effect the reduction.

In Bender's sulfide method the sample is treated with potassium chlorate and hydrochloric acid, and warmed until solution is complete. The free chlorine is removed by aspiration, and the solution is filtered. The filtrate is treated with hydrogen sulfide, and the precipitated mercuric sulfide is collected and weighed in a Gooch crucible.

The potassium iodate method consists simply in the titration of the sample with standard potassium iodate in the presence of hydrochloric acid.

RESULTS OBTAINED.

The potassium iodate method yielded the most consistent and accurate results. Furthermore, it is the simplest and most rapid.

To determine whether the presence of carbohydrates would interfere with the accuracy of this method when applied directly to tablets of mercuric iodide, several mixtures of mercuric iodide with various carbohydrates were assayed. The following results were obtained:

| SAMPLE | | MERCURIC IODIDE FOUND |
|--------|------------------------------------|--------------------------|
| | gram | gram |
| 1: | 0.1 mercuric iodide 1.0 lactose | 0.0999 |
| 2: | 0.1 mercuric iodide 0.5 lactose | 0.0994 |
| 3: | 0.1 mercuric iodide 0.5 starch | 0.1005 |
| 4: | 0.1 mercuric iodide 1.0 sucrose | 0.0994 |

Apparently any error due to these materials is insignificant.

SAMPLE FOR COLLABORATIVE STUDY.

The sample for collaborative study, Sample C, was prepared to simulate commercial tablets of mercuric iodide. The following ingredients in the proportions indicated were thoroughly triturated in a mortar:

¹ *This Journal*, 9, 307 (1920).

| | <i>parts</i> |
|-----------------------|--------------|
| Mercuric iodide | 8 2 |
| Talc | 3 0 |
| Starch..... | 15.0 |
| Sucrose..... | 23.8 |
| Lactose..... | 50 0 |

The mercuric iodide used was a commercial sample of U. S. P. grade.

Assayed by the U. S. P. electrolytic method for mercuric iodide, Sample C was found to contain 8.13 per cent mercuric iodide. This sample was assayed by the three methods, and the following results were obtained:

| METHOD | MERCURIC IODIDE FOUND |
|----------------------------------|--------------------------|
| | <i>per cent</i> |
| I. Rupp | 7 85 |
| | 7 99 |
| II. Sulfide | 8 49 |
| | 8 81 |
| III. Potassium iodate... | 8 07 |
| | 8 12 |

Portions of Sample C, together with the following method, were submitted for collaborative study. Collaborators were instructed to use 1 or 2 grams of the sample, and to report results as percentage of mercuric iodide.

METHOD FOR COLLABORATIVE STUDY.

REAGENT.

Standard potassium iodate.—Dissolve 3 grams of pure potassium iodate, previously dried at about 120°C., in water and dilute to 1 liter. 1 cc. = 0.00637 gram of HgI₂.

DETERMINATION.

Powder and mix a representative number of the tablets. Weigh accurately a sufficient quantity of the powdered material to represent 1-2 grains of mercuric iodide. Transfer to a 300 cc. glass-stoppered Erlenmeyer flask. To the flask add a cooled mixture of 30 cc. of concentrated hydrochloric acid, 20 cc. of water, and about 5 cc. of chloroform. Rotate the flask to disintegrate the powder and dissolve the mercuric iodide.

Titrate with the standard potassium iodate, adding the solution rapidly while rotating the flask. When the iodine which is liberated during the first stage of the reaction has disappeared from the solution, insert the stopper and shake vigorously for about 30 seconds. Continue the titration slowly, shaking thoroughly after each addition, until the iodine color just disappears from the chloroform, marking the end point.

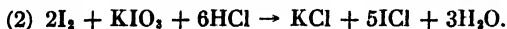
The following equation represents the completed reaction:



The reaction occurs in two stages, in the first of which iodine is liberated. This is represented as follows:



The iodine so liberated reacts with additional potassium iodate as follows:



The following results were reported by the collaborators:

Sample C.

| COLLABORATOR | MERCURIC IODIDE FOUND |
|-----------------|--------------------------|
| | <i>per cent</i> |
| N. E. Freeman | 8.22 |
| | 8.10 |
| Maurice Harris | 8.12 |
| | 8.15 |
| Frank C. Sinton | 8.11 |
| | 8.09 |
| | 8.11 |

COMMENTS BY COLLABORATORS.

N. E. Freeman.—No difficulty was encountered with this method. The end point was sharp and entirely satisfactory. The only question in my mind regarding this method is whether the ordinary C. P. potassium iodate is sufficiently pure so that standardization of this solution may be dispensed with.

Maurice Harris.—No difficulty was encountered with the method, which is short and clean. I might add that the results obtained by this method in the assay of an official sample of mercuric iodide checked very accurately the results obtained by the method of H. O. Moraw¹.

Frank C. Sinton.—The method is very rapid, gives good check results and is otherwise satisfactory.

DISCUSSION.

The results and comments received from the collaborators indicate that the potassium iodate method is satisfactory both in terms of accuracy and in manipulation for the assay of mercuric iodide tablets.

It is of interest to note that the iodate method, slightly modified by Brockman, has recently been found reliable for the determination of the purity of mercuric iodide².

OINTMENTS.

Some preliminary work was done on methods for the assay of mercury compounds in ointments. Unfortunately, no control samples were prepared for study, and no collaborative work was done.

(1) *Calomel Ointment.*—A number of commercial calomel ointments were satisfactorily assayed by a modification of the method for calomel tablets³. This procedure consisted essentially in the removal of the base by dissolving in ether or chloroform and decanting through a filter. The calomel remaining in the flask and on the filter (either paper or asbestos on a Caldwell crucible) is then assayed by the iodometric procedure. The results obtained on two commercial samples of mild mercurous chloride ointment are the following:

¹ *J. Am. Pharm. Assoc.*, 17, 1084 (1928).

² *Am. J. Pharm.*, 101, 596 (1929).

³ *This Journal*, 11, 51 (1928).

| SAMPLE | MILD MERCUROUS CHLORIDE OINTMENTS | |
|--------|-----------------------------------|-----------------|
| | Calomel Declared | Calomel Found |
| | <i>per cent</i> | <i>per cent</i> |
| 1 | 30 | 31 41 |
| 2 | 30 | 28 37 |

(2) *Mercuric Oxide Ointment*.—In the case of ointments of mercuric oxide, the base is not satisfactorily separated from the oxide by organic solvents. However, a modification of the U. S. P. thiocyanate method for stronger mercurial ointment seemed to give satisfactory results for this product.

RECOMMENDATIONS¹.

It is recommended—

(1) That the potassium iodate method be adopted as the tentative method for the assay of mercuric iodide tablets.

(2) That the methods for calomel ointments and mercuric oxide ointments be subjected to collaborative study.

REPORT ON MICROCHEMICAL METHODS FOR ALKALOIDS.

By C. K. GLYCART² (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

During the past four years eleven alkaloids have been studied collaboratively by microchemical methods³.

In accordance with the recommendations made last year, the work was continued with the view to including a systematic description and diagrams of the more important alkaloids.

This year three alkaloids were added for identification: ephedrine, brucine and caffeine.

The work on ephedrine was limited to preliminary tests on the control specimen of ephedrine hydrochloride with the reagents used by Kuen Tsiang and E. D. Brown⁴.

The tests were performed on various dilutions of ephedrine from 1 to 50 and 1 to 1000, as described in their report. Millon's reagent was added to ephedrine in 1-50 dilution, Kraut's reagent to ephedrine in 1-100, platinic chloride, and gold chloride to ephedrine in 1-1000 dilution.

Directions and descriptions for the tests; control specimens of brucine, caffeine and ephedrine; and unlabeled samples containing brucine and caffeine were sent to the collaborators.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 64 (1930).

² Presented by L. E. Warren.

³ *This Journal*, 10, 370 (1927); 11, 353 (1928); 12, 282 (1929).

⁴ *J. Am. Pharm. Assoc.*, 16, 294 (1927).

Sample No. 1 consisted of an aqueous solution of caffeine. Sample No. 2 was an aqueous solution of brucine hydrochloride. The materials for the controls and the tests were products of reputable manufacture and were considered sufficiently pure for the work.

MICROCHEMICAL TESTS FOR ALKALOIDS.

REAGENTS.

A 5 per cent solution of each of the following:

(a) *Potassium iodide.*

(b) *Mercuric chloride.*

(c) *Platinic chloride.*

(d) *Kraut's reagent.*—Dissolve 8 grams of bismuth nitrate in 20 cc. of nitric acid, sp. gr. 1.18. Dissolve 27.2 grams of potassium iodide in a little water and mix. Dilute to 100 cc.

(e) *Millon's reagent.*—Dissolve metallic mercury in an equal weight of strong nitric acid and dilute with an equal volume of water.

PREPARATION OF SAMPLES.

(1) *Controls.*—Dissolve 1 mg. of the pure alkaloidal salt in two drops of water to make an approximately 1–100 solution.

(2) *Alkaloids in compounds.*—Separate the alkaloid in pure form by extracting from ammoniacal solution with a suitable immiscible solvent, and evaporate the solvent. To 1 mg. of the residue add, drop by drop, 0.1 *N* hydrochloric acid, avoiding an excess of acid, and dilute with water, if necessary, to approximately the same alkaloidal concentration as in (1).

(3) *Hypodermic tablets.*—Dissolve a portion of a tablet in water and dilute with water to approximately the same alkaloidal concentration as (1).

IDENTIFICATION.

Place a drop of the alkaloidal solution on a clean glass slide; add a drop of reagent by means of a clean glass rod; and, without stirring or covering, examine under the microscope, using low power. A magnification of 100–150 is suitable. Note the kind of crystals formed, and compare their characteristics with the descriptions given and then with a control.

Characteristic microchemical test for alkaloids.

| ALKALOID | REAGENT | DESCRIPTION OF CRYSTALS |
|----------|-------------------|--|
| Brucine | Potassium iodide | Long masses of transparent rectangular plates, also rosettes of thin plates. |
| | Mercuric chloride | Crystals form in small dense rosettes. |
| Caffeine | Mercuric chloride | Clusters of long radiating needle-shaped crystals. |

The results and comments of the collaborators are as follows:

F. C. Sinton, U. S. F. D. & I. Adm., Chicago, Ill.—

1. Identified as caffeine by mercuric chloride reagent. Long radiating needles were immediately formed.

2. Brucine.—Potassium iodide reagent formed transparent plates generally rectangular, grouped together irregularly in elongated masses. Dense rosettes of thin plates were also formed.

Ephedrine and Millon's reagent 1 : 100:

Thick amorphous precipitate which crystallizes into small clusters of radiating needles. In the 1 : 1000 solution crystals form immediately.

Ephedrine and Kraut's reagent 1 : 100:

Dense precipitate which forms long, red, branching and interwoven needles.

Ephedrine and Gold Chloride 1 : 100:

No precipitate either amorphous or crystalline.

Ephedrine and Platinic Chloride 1 : 100:

No precipitate amorphous or crystalline.

N. E. Freeman, U. S. F. D. & I. Adm., Chicago, Ill.—

1. Identified as caffeine.

2. Identified as brucine.

With both of these samples the crystal formation was rapid, and the crystals formed were characteristic and easily identified.

Further work was done on brucine, various concentrations of the alkaloid being used. It would seem that a rather concentrated solution should be used for this test, preferably between 1-50 and 1-100.

Sufficient time was not available for a complete study of ephedrine. However, the platinic chloride test was attempted on two solutions, both the alkaloid and the hydrochloride being used. The dilutions were 1-100 and 1-500. No crystal formation took place in any instance until the solution had evaporated almost to dryness. The crystals then observed were apparently identical with those reported by Kuen Tsiang and Brown, but on the other hand the same crystals were formed on the spontaneous evaporation of an aqueous solution of the hydrochloride.

It is accordingly believed that the crystals observed were those of ephedrine hydrochloride, the platinic chloride reagent apparently having no effect except that in the case of the solutions of the alkaloid the hydrochloric acid present would naturally form the hydrochloride. However, Kraut's reagent gave characteristic crystals—orange-red to brown needles in interlacing branches.

S. M. Stark, U. S. F. D. & I. Adm., Chicago, Ill.—

Unknown No. 1, Caffeine.—This solution yielded long transparent needle-like crystals with mercuric chloride reagent.

Unknown No. 2, Brucine.—This solution yielded transparent over-lapping plates with potassium iodide reagent.

Caffeine and brucine were easily identified by the methods described.

Ephedrine.

Millon's reagent.—This yielded an amorphous precipitate with a 1 : 100 solution of ephedrine. The precipitate very slowly changed to crystalline form, but the crystals were very small and not sufficiently characteristic in shape to be of use in identifying ephedrine.

Gold Chloride.—There was no crystalline formation when a 1 : 50 ephedrine solution was treated with this reagent.

Platinum Chloride.—There was no crystalline formation when a 1 : 50 ephedrine solution was treated with this reagent.

Kraut's reagent.—This reagent reacted with a 1 : 100 ephedrine solution to yield a mass of opaque, bushy crystals.

DISCUSSION.

The findings of the collaborators show that the microchemical tests for brucine and caffeine are satisfactory. The preliminary work on microchemical tests for ephedrine performed by the associate referee indicates that Kraut's reagent and Millon's reagent yield characteristic crystals; however, the tests with platinic and gold chloride are not suitable. These tests were confirmed by the collaborators.

RECOMMENDATIONS¹.

It is recommended—

(1) That the microchemical tests for brucine and caffeine be adopted as tentative.

(2) That further study be made on tests for identification of ephedrine, also aconitine, physostigmine, yohimbine and arecoline with a view to tentative adoption.

No report on terpin hydrate was given by the associate referee.

REPORT ON SANTONIN.

By HENRY M. BURLAGE² (School of Pharmacy, Purdue University, Lafayette, Ind.), *Associate Referee*.

A previous collaborative investigation³ conducted by the present associate referee shows that in general the existing gravimetric methods are unsatisfactory. The results on a non-fatty mixture containing santonin were far below the amount actually present in the sample, and the values for fatty mixtures were so varied that they were not even considered for study.

It was decided to spend no more time studying other gravimetric methods or devising new gravimetric methods, but to make a study of existing volumetric methods and if possible to ascertain their applicability to the assay of santonin in such types of mixtures as mentioned above. This report is an outline of such a study. Time did not permit the associate referee to carry on a collaborative study; however, it is hoped that the methods presented are worthy of consideration as tentative methods until, if deemed desirable, a satisfactory collaborative study can be carried out.

PREPARATION OF SAMPLES.

Samples were prepared as directed in the previous report³. Sample A is a non-fatty mixture containing 2.94 per cent of santonin, and

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 64 (1930).

² Presented by L. E. Warren.

³ *This Journal*, 12, 284 (1929).

in every case 5.0000 gram samples were weighed out. Sample AA' is a non-fatty mixture of the ingredients of Sample A but containing no santonin; in every case 4.8529 grams of the sample was taken, which is equivalent to the amount of ingredients other than santonin in 5.0000 grams of Sample A.

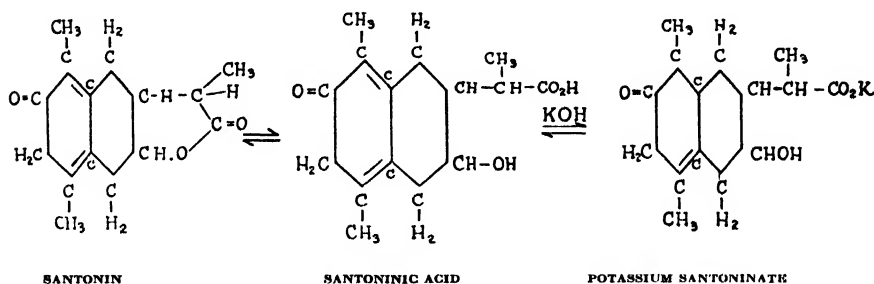
Sample B is a fatty mixture containing $9.39 \pm$ per cent of santonin, and in all cases but one (2.0000 grams) 1.5000 grams of sample was employed; in case of sample BB', 1.3574 grams of sample was used; this weight is equivalent to the amount of ingredients other than santonin in 1.5000 grams of Sample B.

With samples of the fatty mixtures it will be noted that less uniform results were obtained. This is probably due to the fact that these samples could not be prepared in an absolutely dry condition, and, consequently, uniformity of samples could not be assured.

METHODS.

I. THE KARIYONE AND KIMURA METHOD (MODIFIED).

Kariyone and Kimura¹ modified the Katz method² for the determination of santonin in santonica. The method employed by these investigators determines the santonin in the final purified chloroform extract by volumetric methods instead of employing the usual gravimetric method of weighing the residue after heating to constant weight. This volumetric determination depends upon the fact that santonin is an inner anhydride of santoninic acid, and as such may be converted into this acid and finally into the alkali salts of this acid by treating with alkalis according to the following reactions:



Non-fatty Mixtures.

Procedure A:

Samples equivalent to 0.1–0.2 gram of santonin are accurately weighed and then dissolved in 30 cc. of boiling alcohol. The solution is made neutral to phenolphthalein and an excess of 20 cc. (accurately measured)

¹ *J. Pharm. Soc. Japan*, No. 405, 927; *Pharm. Weekbl.*, 58, 1299 (1921); *Yearbook Am. Pharm. Assoc.*, 10, 274 (1921).

² *Arch. der Pharm.*, 238, 100 (1900); *Centrbl.*, 1, 877 (1900).

of 0.1 *N* KOH is added; the solution is digested under a reflux on a water bath for 20 minutes and then titrated with 0.1 *N* sulfuric acid. A blank of 30 cc. of neutral alcohol and 20 cc. of the standard alkali is treated in like manner.

Procedure B:

Tenth normal HCl was used instead of 0.1 *N* sulfuric acid, and Procedure A was followed.

Procedure C:

Samples were dissolved in 30 cc. of hot absolute alcohol and filtered. The filter and its contents were washed with three 5 cc. portions of hot absolute alcohol, and the alcoholic solution was treated as in Procedure A.

Procedure D:

Procedure A was modified as follows: Samples were treated with 30 cc. of warm neutral alcohol, and the mixture was filtered by the aid of suction through a Gooch crucible provided with an asbestos mat; the residue was washed with three 10 cc. portions of warm neutral alcohol. The filtrate was rendered neutral to phenolphthalein by the addition of approximately 0.1 *N* KOH; an excess of 25 cc. of alkali (exactly measured) was added, and the solution was digested on a water bath under a reflux condenser for one-half hour. It was then titrated with 0.1 *N* HCl. In the same manner the following blanks were run: (1) 25 cc. of the alkali solution; (2) 60 cc. of neutral alcohol (equal to the total amount of alcohol used in dissolving and washing the sample); and (3) Sample AA' representing the true amount of ingredients other than santonin in Sample A.

Procedure E:

The method described in D was repeated; absolute alcohol was used, and the titration was carried out on the hot solution.

TABLE 1.

Modified Kariyone and Kinura method (non-fatty mixtures).

| PROCEDURE A | B | C | D | | | E | |
|------------------|-----------------|-----------------|------------------|----------------------|----------------------|------------------|----------------------|
| | | | Uncor- rected | Corrected for (2) | Corrected for AA' | Uncor- rected | Corrected for AA' |
| <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 3.86 | 3.84 | 4.99 | 3.26 | 3.08 | 2.93 | 3.30 | 2.98 |
| 3.85 | 4.04 | 4.99 | 3.30 | 3.10 | 2.94 | 3.27 | 2.95 |
| 3.89 | 4.09 | | | | | 3.11 | 2.90 |
| 3.76 | 4.09 | | | | | 3.11 | 2.90 |
| Average: 3.84 | 4.02 — | 4.99 | 3.28 | 3.09 | 2.94 — | 3.20 — | 2.93 |

COMMENTS.

(1) Procedure A gives consistent results which are much too high, indicating that the other ingredients in the sample are soluble and react with the alkali.

(2) Most procedures direct the use of hydrochloric acid and it was thought advisable to try Procedure A, using this acid instead of sulfuric acid. The results are still consistently high, indicating other soluble materials which react with the alkali.

(3) In order to eliminate the error arising in Procedures A and B due to solubility of ingredients other than santonin, absolute alcohol was used instead of 95 per cent alcohol (C). Results obtained were even higher than those of A and B.

(4) Method D is satisfactory for non-fatty mixtures if a correction is applied, which is obtained by determining the amount of alkali required by the ingredients other than santonin (Sample AA'), which are present in 5.0000 grams of Sample A.

(5) Procedure D is applicable if ordinary or absolute alcohol (hot or cold) is used, and the titration is carried out on the hot or cold solution, provided the results are corrected for AA', the amount of ingredients other than Santonin in Sample A reacting with the alkali.

Fatty Mixtures.

Procedure C. See above.

Procedure F.

The sample was washed with three 10 cc. portions of petroleum ether (saturated with santonin) and filtered, and the fat-free(?) residue was treated with 30 cc. of neutral alcohol. The solution was made neutral to phenolphthalein and then 25 cc. of 0.1 *N* (approximately) KOH was added and the method given under D was followed. A blank with Sample BB' (1.3574 grams), which contains the ingredients other than santonin in 1.5000 grams of Sample B, was treated in the same manner.

Procedure G:

Neutral absolute alcohol was used instead of neutral alcohol as in F.

Procedure H:

F was repeated, hot absolute alcohol being used.

TABLE 2.
Modified Kariyone and Kimura method (fatty mixtures).

| PROCEDURE C | F | | G | | H | |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------------------|
| 2.0000 grams | Uncorrected | Corrected | Uncorrected | Corrected | Uncorrected | Corrected |
| <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | |
| 10 85 | 8 83 | 5 47 | 9 73 | 8 34 | 8 88 | no correction necessary |
| 10 85 | 8.34 | 5 96 | 10 01 | 8 54 | 9 06 | |
| Average: 10.85 | 8 59— | 5.72— | 9 87 | 8 44 | 8 93 | |

COMMENTS.

(1) Procedure C also gives high results with fatty mixtures, showing that other ingredients, which react with the alkali, are dissolved.

(2) By Procedure F the end point was difficult to determine with fatty mixtures since a brown solution was obtained. The results were consistently low and even more so when corrected for BB'; BB' was a freshly prepared sample; for subsequent determinations another sample of BB' was used.

(3) Procedures F and G show that the method outlined in D is not applicable to fatty mixtures.

II. LANGER'S METHOD (MODIFIED).

Recently Langer¹ proposed the following method for the titration of santonin in pharmaceutical preparations:

(A) *Tablets*.—Extract tablets with benzene to remove phenolphthalein, and if fats are present, with petroleum ether saturated with santonin (0.05 gram per 100 cc.). Dissolve the santonin (0.2–0.3 gram) in 20 cc. of alcohol and 20 cc. of 0.05 *N* alcoholic KOH, reflux by immersion in a water bath for $\frac{3}{4}$ hour, and titrate with 0.05 *N* HCl. Toward the end of the titration a bright yellow color develops. The alcohol must be neutralized or a correction applied.

(B) *Confections*.—Rub up the kernels with washed sand, dry to 80°C. to remove the esters, shake the cooled powder with petroleum ether saturated with santonin, decant, and extract with two like portions of petroleum ether before filtration; press the filter residue between two filters and then extract with benzene. After repeated shaking, allow to stand for 14 hours, pass through a pledget of cotton, evaporate a weighed aliquot (containing about 0.15–0.16 gram of santonin) of the filtrate to dryness at 80°C. and weigh the residue, which is washed with petroleum ether (saturated with santonin), and then titrate as in (A) above.

Langer's method is long and tedious, and because of the many extractions and filtrations is subject to criticism. Accordingly, the following modified procedures were tried:

Procedure A:

Hot benzene was used, and all filtrations were made by suction on a Gooch crucible provided with an asbestos mat.

Procedure B:

Samples were treated directly with 20 cc. of neutral alcohol. The solution was made neutral to phenolphthalein, 25 cc. of 0.05 *N* KOH was added, and the solution was then digested on a water bath for $\frac{3}{4}$ hour under a reflux and titrated with 0.05 *N* HCl.

Procedure C:

Procedure B was repeated, and the alcohol solution was filtered.

Procedure D:

Samples were dissolved in 30 cc. of boiling alcohol and filtered. The filter was washed with two 10 cc. portions of hot alcohol. The alcoholic

¹ *Apoth. Ztg.*, 43, 815 (1928); *C. A.*, 22, 3488 (1928).

solution was neutralized with the base, phenolphthalein being used as an indicator; 20 cc. of 0.05 *N* alcoholic KOH was added, and the mixture was refluxed on a water bath for 20 minutes and then titrated with 0.05 *N* HCl; a blank consisting of 50 cc. of neutral alcohol and 20 cc. of the base was run in like manner.

Procedure E:

The samples were extracted with 15, 10, 5 and 5 cc. portions of benzene, successively; each portion was filtered by suction through a Gooch filter provided with an asbestos mat; each portion was drawn completely through the filter before the next portion was added. The benzene was distilled off, and the residue was dissolved in 30 cc. of boiling alcohol and neutralized; 25 cc. of approximately 0.1 *N* KOH was added, and the mixture was digested on a water bath under a reflux for 20 minutes; the hot solution was titrated, 0.1 *N* HCl being used.

Procedure F:

Procedure E was repeated, and the boiling alcohol was replaced with 30 cc. of aldehyde-free alcohol.

Procedure G:

Procedure E was repeated, and neutral alcohol was used to dissolve the residue from the benzene extract.

Procedure H:

Procedure E was repeated, and hot benzene and neutral aldehyde-free-alcohol were used.

Procedure I:

Langer's method was modified for Sample B. The sample was first extracted with 10, 10, 10, 5 and 5 cc. portions of petroleum ether (saturated with santonin) to remove fatty materials. The santonin was extracted from the fat-free(?) residue by treating with successive portions of 15, 10, 5 and 5 cc. of benzene; the benzene was evaporated; the santonin residue was dissolved in 25 cc. of aldehyde-free alcohol; and the usual procedure was followed.

Procedure J:

The samples were extracted with 10, 10, 10, 5 and 5 cc. portions of petroleum ether saturated with santonin (if the sample is fat-free this step may be omitted). All portions were filtered by suction to complete dryness before following with another portion through a Gooch crucible provided with an asbestos mat. The residue in the container and the crucible was then extracted with 15, 10, 5 and 5 cc. of hot benzene, each portion being filtered as before. The benzene extract was evaporated in a tared flask, and the residue of santonin was dried to constant weight at 100°C. The weight of the material in the flask is equal to the weight of the santonin. As a check, this residue was dissolved in 25 cc. of aldehyde-free alcohol by warming and neutralized; 25 cc. of 0.1 *N*

(approximately) KOH solution was then added, and the mixture was digested on a water bath under a reflux for $\frac{1}{2}$ hour and then titrated hot with 0.1 N HCl. A blank consisting of 25 cc. of aldehyde-free alcohol and 25 cc. of the base was also run in the same manner.

TABLE 3.
Modified Langer's method (non-fatty mixtures).
(Results expressed in percentage.)

| PROC.: A | B | C | D | E | F | G | H | J GRAV. | J VOL. |
|------------------|------|------|-------|-------|------|------|-------|------------|-----------|
| 3.16 | 3.45 | 2.30 | 2.70 | 3.04 | 2.79 | 2.85 | 2.90+ | 2.82 | 2.89 |
| 3.09 | | 2.48 | 2.69 | 3.01 | 2.79 | | 2.86 | 2.97 | 2.91 |
| 3.08 | | | | | | | | | |
| Average: 3.11 | 3.45 | 2.39 | 2.70— | 3.03— | 2.79 | 2.85 | 2.88+ | 2.90— | 2.90 |

TABLE 4.
Modified Langer's method (fatty mixtures).

| PROCEDURES: A | I | J—GRAV. | J—VOL. |
|-------------------|-----------------|-----------------|-----------------|
| <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 10.00 | 9.23 | 9.72 | |
| 10.16 | 9.65 | 9.83 | 9.82 |
| Average: 10.08 | 9.44 | 9.78 | 9.82 |

COMMENTS.

1.—In Procedure C the results are low. It may be that the solvent was not entirely removed from the ingredients that were insoluble and the residue was not washed.

2.—In Procedure D results are low, probably for the same reason given for Procedure C.

3.—In Procedure E the end point was difficult to determine, which might account in part for the high results obtained.

4.—When aldehyde-free alcohol was used, as in the case of Procedure F, a satisfactory end point was obtained. The results were low, indicating incomplete extraction.

5.—Procedure J is entirely satisfactory for non-fatty mixtures, but the results are not so encouraging in the case of fatty mixtures. It seems almost impossible to remove entirely the fatty constituents which might react with the base to bring about high results. However, as mentioned above, since this mixture cannot be dried completely during its preparation and thus may not be uniform, the discrepancy arising is in part explained. The results for fatty mixtures by this method are the most

satisfactory that have been obtained, and even though they are consistently high, the method will give some indication of the relative amounts of santonin in such mixtures.

III. PALKIN'S METHOD.

Palkin¹ suggested a method for the assay of santonin in *Santonica* which might be applied to the above types of mixtures. Some preliminary experiments were tried in an attempt to modify this method for the determination of santonin in fatty and non-fatty mixtures; complete details of the method are not given and time did not permit the development of an entirely new method involving the principles put forth by Palkin.

SUMMARY.

Previous results show that most of the existing gravimetric methods are unsatisfactory, especially in the case of fatty mixtures containing santonin.

Volumetric methods have been tried, including modifications of the Kariyone and Kimura method and Langer's method.

The Kariyone and Kimura method modified for non-fatty mixtures gives accurate results if a blank representing the ingredients other than santonin is run according to the modified method and the results on the original sample are corrected for the blank. The modified method does not give satisfactory results with fatty mixtures.

The Langer method has been modified with encouraging results. The modified method gives very satisfactory results gravimetrically which check well with volumetric results. The results are particularly satisfactory in the case of non-fatty mixtures. A blank is not necessary. In the case of fatty mixtures the results are consistently high, indicating the solution of substances other than santonin which react with the alkali. The method, however, will give some indication of the relative amount of santonin in such fatty mixtures.

RECOMMENDATIONS².

It is recommended—

(1) That the method outlined under Procedure D (Method I above) be adopted for non-fatty mixtures containing santonin whose exact composition is known.

(2) That the method of Langer as modified under Procedure J be adopted for the assay of santonin in non-fatty mixtures, and that this method be adopted tentatively for the assay of santonin in fatty mixtures.

(3) That in case the recommendations as given in 1 and 2 are not adopted, a collaborative study be conducted at an early date.

¹ *This Journal*, 9, 328 (1926).

² For report of Subcommittee B and action of the association, see *This Journal*, 13, 65 (1930).

(4) That if the results obtained for fatty mixtures by Procedure J are considered to be too high for the adoption of Procedure J as a tentative method for the assay of santonin in fatty mixtures, further studies be given to this modified method, which promises much since it can be used as a gravimetric as well as a volumetric method and does not require a blank determination necessitating the knowledge of the exact composition of the preparation before proceeding.

(5) That future investigations be extended to a study of colorimetric methods as well as new methods.

(6) That the Palkin method be studied more fully.

(7) That the methods of the assay of santonin in the crude drug be studied.

REPORT ON ETHER.

By WM. F. KUNKE¹ (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

The work during the past year was devoted mainly to experimentation with modifications of the Somogyi² method for the quantitative determination of ether in ether-alcohol-water solutions with a view to working out a simplified, equally accurate and more practical procedure. No collaborative work was done.

To give a better understanding it may not be amiss to relate the salient points of the Somogyi method and of the modifications tried out by previous associate referees. The Somogyi method directs the introduction of the ether and alcohol vapors into a stream of dry air, with which the vapors are bubbled by distillation and aspiration successively through sulfuric acid (1 + 3) and through a mixture of concentrated sulfuric acid and normal potassium dichromate solution, equal parts by volume. The sulfuric acid (1 + 3) contained in the first tube retains the alcohol, while the ether vapors pass through into the second tube where the ether is oxidized. The excess normal dichromate solution in the second tube is titrated, and from the dichromate solution consumed the amount of ether in the sample may be easily calculated. Somogyi heated the flask containing the ether-alcohol sample, thereby distilling both the ether and alcohol, whereas the associate referees for 1927 and 1928 heated the ether sample just sufficiently to expel the stopper from the glass-stoppered capsule in which the ether sample had been weighed.

The associate referee for 1927 modified the apparatus by providing both tubes, I and II, with jacket tubing by means of which No. I may be kept at the temperature of 50°C. and No. II at the temperature of

¹ Presented by H. Runkel.

² *Z. angew. Chem.*, 39, 280 (1926).

ice water. Last year's associate referee simplified the above modification by eliminating the jacket tubing for both tubes. Sulfuric acid (1 + 2) was substituted for sulfuric acid (1 + 3) used to retain the alcohol vapors, no doubt with a view to greater efficiency.

Somogyi weighed the ether or the anhydrous alcohol-ether sample in a sealed glass tube, while the former associate referees used a glass-stoppered capsule to weigh the ether or ether-alcohol-water samples.

The associate referee for 1927 reported that repeated trials demonstrated that the Somogyi method is not dependable for such mixtures as may be obtained by distillation from medicinal preparations.

Somogyi, as did the previous associate referees, reported good results, considering the extreme care with which such a volatile substance as ether must be handled.

This year experiments with various modifications were carried out.

First: Applied heat was not used.

Second: The rate at which the bubbles were passed through the absorption train by aspiration was increased, in an effort to shorten the time necessary for complete aspiration.

Third: Ether or ether-alcohol or ether-alcohol-water samples containing high percentages of ether or alcohol do not simulate samples containing ether which are most likely to be met with in the laboratory. Therefore, solutions of ether-water or ether-alcohol-water containing very small known quantities of ether were made up, and aliquots of these samples were used for the determination of ether.

Fifteen weighings made of ether drained from a 10 cc. pipet into a weighed 25 cc. glass-stoppered volumetric flask gave a range of 6.998–7.0155 grams, or a maximum variation of -0.1 per cent and $+0.16$ per cent from the average weight of 7.0045 grams. The average weight found was used as the weight of the ether drained from the pipet when ether-water or ether-alcohol-water samples containing small quantities of ether or alcohol or both were made up.

Fourth: Each of the two tubes used in the Somogyi method is 70 cm. long and has a 2.5 cm. bore. In place of each of these tubes, two 50 cc. graduated cylinders were used, each cylinder being about 32 cm. tall and having an inside diameter of 1.5–2 cm. This modification in apparatus was made for three reasons: the cylinders are easily obtained, it is believed that an amount of sulfuric acid (1 + 2) in two cylinders equal to that used in one tube will more efficiently retain or even retain more alcohol, and also the sulfuric acid-potassium dichromate solution in two cylinders will be more efficient than the same amount in one tube.

SUMMARY.

The experimental work of this year indicates that the modifications suggested give promise of working out a simplified, equally accurate and more practical procedure for ether samples.

RECOMMENDATIONS¹.

It is recommended that experimental work on the associate referee's various modifications of the Somogyi method for the determination of ether and other modifications be continued the coming year with special reference to medicinal preparations, and that if possible the method with modifications be studied collaboratively.

REPORT ON BIOASSAY OF DRUGS.

By WM. T. McClosky (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

At the 1928 meeting of the association, the associate referee read a contributed paper on "Commercial Posterior Pituitary Powders and Whole Pituitary Powders", by the associate referee and J. C. Munch. In this paper it was suggested (1) that the method of the U. S. P. X for the assay of liquor pituitarii be made a tentative method of the A. O. A. C. for the assay of pituitarium, U. S. P. X, as well as for the assay of desiccated whole pituitary powder; and (2) that the following standards for physiological activity be adopted: Pituitarium, U. S. P. 50 per cent of the activity of the U. S. P. X official standard posterior pituitary powder; desiccated whole pituitary powder, 5 per cent of the activity of the U. S. P. X official standard posterior pituitary powder.

Further work by the referee and M. R. Thompson of the Pharmacological Laboratory has confirmed the results of McClosky and Munch. In addition, the results have been checked on the blood pressure of anesthetized dogs. It is therefore recommended¹ that these methods be made official.

Thompson has carried on an extensive investigation of the pharmacology of ergot, in which he has suggested ways to eliminate some of the confusion existing at the present time in regard to the bioassay of its galenicals.

No report on fluidextract of ginger was given by the associate referee.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 65 (1930).

REPORT ON EPHEDRA.

By C. K. GLYCART and A. E. PAUL¹ (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referees*.

The work on ephedra and its alkaloids was continued in accordance with the recommendation made at the last meeting².

Ephedrine possesses characteristics markedly different from other alkaloids. Moraw³ pointed out that gravimetric methods were not suitable because the alkaloidal residue loses in weight, and stated that a white sublimate, either the alkaloid itself or a decomposition product, continues to settle in the insides of desiccators in which it is dried.

Preliminary to the collaborative work, the associate referee tried the double extraction procedure as used in the assay of apomorphine tablets on samples of ephedrine hydrochloride, but the method was rejected because low results were obtained.

For the work this year, a commercial product of ephedrine hydrochloride was considered sufficiently pure for use, since it responded to the theoretical figures for melting point and specific rotation.

Directions for analysis and specimens of a mixture containing one part of ephedrine hydrochloride and two parts of milk sugar were sent to the collaborators; 0.4 gram of the mixture was directed for each determination.

EPHEDRINE IN TABLETS.

REAGENTS.

- (a) *Strong ammonium hydroxide.*
- (b) *Washed ether.*—Shake equal volumes of ethyl ether and water in a separatory funnel and discard the aqueous layer.
- (c) *Bromthymol blue indicator.*—0.04 per cent alcoholic solution.
- (d) *Distilled water.*—Free from carbon dioxide.
- (e) *Sulfuric acid.*—0.02 N.

PREPARATION OF SAMPLE.

To determine the average weight per tablet, count and weigh 100 tablets or a number representative of the lot.

DETERMINATION.

Weigh not less than 20 tablets. Powder in a mortar, weigh accurately a quantity equal to about 2 grains of the alkaloidal salt, and transfer to a separatory funnel. Dissolve in the minimum quantity of water, then add 5 cc. of strong ammonium hydroxide.

Extract the solution with 30 cc. of washed ether. Transfer the aqueous layer to a second separatory funnel. Wash the ether extraction with 1 cc. of water, adding the washings to the main aqueous solution. Swirl the ether in order to remove water adhering to the side of the separatory funnel. After all the water has been removed, filter into a beaker through a pledget of cotton wet with ether inserted in a small funnel.

¹ Presented by L. E. Warren.

² *This Journal*, 12, 290 (1929).

³ *J. Am. Pharm. Assoc.*, 17, 431 (1928).

Repeat the extraction with liberal portions of ether at least four times, or until the alkaloid is removed completely, washing each portion with 1 cc. of water. Evaporate the ether to a volume of 10 cc. on a steam bath with moderate heat before a fan (see "Discussion"). Finish the evaporation without heat to prevent loss by volatilization of ephedrine.

Dissolve the residue in 2 cc. of neutral alcohol without heat, add 0.5 cc. of bromthymol blue indicator, and titrate with 0.02 *N* sulfuric acid to a green-yellow end point, finally diluting with 20 cc. of carbon dioxide-free water. (A standard comparator color of pH 6.5 is convenient for a control.) 1 cc. of 0.02 *N* acid = 4.03 mg. of ephedrine hydrochloride, equals 4.28 mg. of ephedrine sulfate, and equals 3.30 mg. of ephedrine.

QUALITATIVE TESTS FOR EPHEDRINE BY COPPER SULFATE—SODIUM HYDROXIDE.

To 1 cc. of solution containing at least 5 mg. of ephedrine, add 0.1 cc. of 10 per cent copper sulfate and 1 cc. of 20 per cent sodium hydroxide solution. Shake with 2 cc. of ethyl ether.

A purple color extracted by ether indicates ephedrine or pseudo ephedrine.

| | | MELTING POINT °C. | ALPHA 20/D | SOLUBILITY |
|----------------------------|-----------------------|----------------------|--------------|------------|
| Ephedrine | $C_{10}H_{15}NO$ | 39-40 | Laevo -6.3 | Water |
| Pseudo ephedrine | $C_{10}H_{15}NO$ | 117-118 | Dextro -51.2 | Cold water |
| Ephedrine hydrochloride | $C_{10}H_{15}NO\ HCl$ | 214-216 | Laevo -35.5 | Water |

| COLLABORATOR | EPHEDRINE HYDROCHLORIDE IN TABLETS per cent |
|---|--|
| H. McCausland Abbott Laboratories North Chicago, Ill. | 32.1 |
| E. O. Eaton | 32.14 |
| F. D. I. Administration San Francisco, Calif. | 31.68 31.44 |
| F. C. Sinton | 31.15 |
| F. D. I. Administration Chicago, Ill. | 31.05 |
| N. E. Freeman | 30.7 |
| F. D. I. Administration Chicago, Ill. | 30.6 |

COMMENTS.

N. E. F.—The method used was that submitted with the sample, and no difficulty was encountered except in getting rid of the moisture remaining in the beaker after the evaporation of the ether. It was also noticed that the alkaloid had a tendency to creep quite badly and it was necessary to wash this down with ether a couple of times during the evaporation. After practically all the ether had evaporated, the beakers were left for approximately 2 hours before the fan in order to remove any traces of ammonia from the moisture remaining.

The following results were obtained by J. B. Williams, Parke, Davis & Co., Detroit, Mich.:

| per cent | per cent |
|----------|----------|
| 28.22 | 30.34* |
| 29.74 | 30.04 |
| 30.44 | 30.04 |
| 30.54 | 30.34 |

* 0.5 cc. strong ammonia used instead of 5 cc.

J. B. W.—Three portions of the mixture, 0.2, 0.4, and 0.2 gram, were also assayed by extraction with ether, but sodium hydroxide was used instead of ammonia, a measured excess of 0.1 *N* acid was added to the ether solution before evaporation, and the excess acid was titrated with 0.02 *N* NaOH, methyl red indicator being used; 33.33, 32.82, and 33.26 per cent was found.

DISCUSSION.

It is evident that losses of alkaloid occur during the final stages of evaporating the ether and water of condensation.

Freeman stated that the beakers were left for approximately 2 hours before the fan in order to remove any traces of ammonia from the moisture remaining.

Williams reports high results by using sodium hydroxide instead of ammonia. He also titrates with a measured quantity of 0.1 *N* acid added to the ether before complete evaporation, and the excess is titrated with 0.02 *N* sodium hydroxide. It is believed that this procedure of titration should be included in the method.

EPHEDRA.

Last year a method for the assay of ephedra was submitted for collaborative study. The analytical data of four of the collaborators are summarized as follows:

Alkaloids of ephedra, per cent (average).

| MCCAUSLAND | WILLIAMS | MORAW | SINTON |
|------------|----------|-------|--------|
| 0 995 | 1.02 | 0 98 | 1.01 |

The results were considered satisfactory, but inasmuch as ephedra is a new topic it was thought that certain modifications suggested in the comments of the collaborators should be studied before the assay was recommended to the association. Accordingly, the work this year was devoted to the length of time required for maceration of the powdered drug in the ether-chloroform solvent. By making comparative assays, the associate referee found that maceration for 4 hours is sufficient, as pointed out by McCausland.

RECOMMENDATIONS¹.

It is recommended—

(1) That the ephedra assay reported last year be adopted as tentative after the following changes have been made:

Change the direction, "and shake the mixture intermittently for 2 hours; then allow to macerate 4 hours", to read, "Allow to macerate at least 4 hours, shaking occasionally".

(2) That methods for the assay of ephedrine in pharmaceuticals be further studied.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 65 (1930).

REPORT ON THYMOL.

By LESLIE HART (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*.

The determination of thymol in antiseptic solutions is somewhat complicated owing to the presence of methyl salicylate and to the essential oils that are frequently found in antiseptic solutions containing this drug. *Liquor antisepticus*, N. F., on which the preliminary work necessary for this study was done, contains 0.1 gram of thymol per 100 cc. Interfering constituents are: eucalyptol, 0.5 cc. per 100 cc.; methyl salicylate, 0.12 cc. per 100 cc.; menthol, 0.1 gram per 100 cc.

Sherk¹ states that thymol may be removed from alkaline solution by repeated extractions with ether. Following this suggestion, the associate referee extracted 50 cc. of an alkaline thymol solution (containing 0.1 gram of thymol) four times with 25 cc. portions of ether, evaporated the ether spontaneously, and dissolved the residue in 5 per cent sodium hydroxide solution. This solution was titrated with 0.1 *N* bromine solution according to the method submitted to the association last year². The average of four titrations was 0.094 gram of thymol, which showed a slight loss. The experiment was then repeated by the addition of 5 cc. of 0.5 *N* alcoholic potash solution to the ether extract before evaporation. The average of five titrations was 0.097 gram of thymol.

These experiments were used by the associate referee as a basis for preliminary work on a method for the determination of thymol in *liquor antisepticus*, N. F.

The method is as follows:

METHOD TENTATIVELY DEVELOPED FOR DETERMINATION OF THYMOL IN ANTISEPTIC SOLUTIONS.

Pipet 50 cc. of the sample into a platinum dish, add 5 cc. of 25 per cent sodium hydroxide solution, and place on the steam bath to evaporate the alcohol. Transfer the residue to a 125 cc. separatory funnel, dilute to about 75 cc., and extract with petroleum ether to remove essential oils. Wash the petroleum ether extract once with 10 cc. of 5 per cent sodium hydroxide and add washings to the aqueous layer.

Extract the combined aqueous solution and washings four times with ether, using 25, 20, 20 and 15 cc. Combine the ether extracts into a 250 cc. beaker, add 10 cc. of 0.5 *N* alcoholic potash solution, and evaporate at gentle heat. Dilute to 25 cc., add 20 cc. of hot 1 + 1 hydrochloric acid, and titrate immediately with 0.1 *N* bromine solution, using methyl orange as an indicator, according to Method I, previously described³. Report results as grams of thymol per 100 cc.

W. F. Kunke, of the Chicago Station drug laboratory, kindly made a few determinations as a check of the method.

The following results were obtained on a solution containing 0.10 gram of thymol per 100 cc.:

¹ *Am. J. Pharm.*, 93, 115, 207 (1921).

² *This Journal*, 12, 54 (1929).

³ *Ibid.*, 55.

| | |
|-------|-------|
| 0.103 | 0 095 |
| 0.093 | 0 097 |
| 0 092 | 0 092 |
| 0.090 | |
| 0.096 | |

It is recommended that this study be continued¹.

REPORT ON MENTHOL.

By F. L. ELLIOTT (U. S. Food, Drug and Insecticide Administration, Baltimore, Md.), *Associate Referee*.

The method for the determination of menthol submitted to collaborators last year² gave uniform but slightly high results. This was thought to be due to the acetic anhydride remaining in the washed acetylyzed oil. It was recommended that further study be made of the difficulties in removing acetic anhydride from the acetylyzed oil.

In order to obtain a uniform sample, approximately 140 cc. of acetylyzed oil was prepared from U. S. P. menthol by the original method, the same proportion of acetic anhydride being used. This unwashed acetylyzed oil was divided into four portions and washed to remove the excess of acetic anhydride by the following methods:

(1) Washed with 6 per cent sodium carbonate solution in separator until alkaline to phenolphthalein, as in original method.

(2) Boiled in reflux with two volumes of water for 15 minutes. The water was removed, and the oil was cooled and washed with sodium carbonate solution as directed above until alkaline.

(3) Boiled in reflux with two volumes of water as in (2), except that this operation was repeated four times with separate portions of water; the oil was cooled and washed with sodium carbonate until alkaline.

(4) Washed with 6 per cent sodium carbonate as in (1) until alkaline; the oil was refluxed with two separate portions of water and rewashed with sodium carbonate solution after cooling.

The acetylyzed oil washed by these different methods was dried over anhydrous calcium chloride and filtered, and menthol was determined by the original method. The percentages found were as follows:

| METHOD | per cent | |
|--------|----------|-------|
| 1 | 100 3 | 100 2 |
| 2 | 100 9 | 100 9 |
| 3 | 100 3 | 100.5 |
| 4 | 100.5 | 100 7 |

It is evident from these results that the high percentages obtained cannot be due to the presence of free acetic anhydride remaining in the acetylyzed oil¹.

No report on bromides-chlorides was given by the associate referee.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 66 (1930).

² *This Journal*, 12, 300 (1929).

REPORT ON CHENOPODIUM OIL.

THE DETERMINATION OF ASCARIDOLE IN CHENOPODIUM OIL.

By L. B. BROUGHTON, *Associate Referee*, and G. S. WEILAND (Chemistry Department, University of Maryland, College Park, Md.).

Chenopodium or American wormseed oil has acquired special importance during the last ten years from its use in the campaign against hookworm.

It is now known from the work of Schimmel & Co.¹ and Nelson² that the chief components of the oil are (a) ascaridole (Formula I), which is present to the extent of 60–75 per cent; and (b) a mixture of terpenes with *p*-cymene, *l*-limonene and probably *a*-terpinene, collectively known as the hydrocarbon fraction.

The work of Smillie and Pessoa³ no longer leaves any doubt that the organic peroxide ascaridole is the sole component of the oil that exhibits anthelmintic action against hookworm and roundworm, the parasites for which the oil is generally used.

Since the establishment of this fact, many attempts have been made to develop a means of ascertaining the quantity of ascaridole present in an oil. Color reactions that take place on heating chenopodium oil with other substances have been described and recommended for qualitative information. Wirth⁴, in 1920, stated that a 50 per cent solution of potassium hydroxide in 50 per cent aldehyde-free alcohol was suitable for a microchemical reagent for chenopodium oils. Langer⁵, in 1921, proposed the use of phenolphthalein, taking advantage of the red color produced. As a quantitative measurement, this color reaction is only fairly satisfactory. The intensity of the red coloration is proportional to the ascaridole content only if it is heated uniformly at a temperature of 155°C. With oils of at least 60 per cent ascaridole content, the experimental conditions are easy to check, but not with those of lower content. The lower boiling portions prevent the heating of the oil to this temperature in a given time, so that the red color is not apparent at first, and if heating is continued the lower fractions boil off. Knaff-Lenz and Hofmann⁶ attempted to perfect a biological method, using worms, fish and mice, with unsatisfactory results. These authors, however, have proposed a color reaction with hydrochloric acid that shows some merit.

Of the quantitative methods, two have been proposed. In 1921 Nelson⁷ published an assay method based on the fact that the hydro-

¹ Schimmel & Co., Report, April, 1908.

² *J. Am. Chem. Soc.*, 33, 1404 (1911); 34, 351 (1913); 42, 1204 (1920).

³ *J. Pharmacol.*, 24, 359 (1924).

⁴ *J. Am. Pharm. Assoc.*, 9, 127 (1920).

⁵ *Pharm. Ztg.*, 66, 191 (1921).

⁶ *Arch. Pharm.*, 2, 117 (1920).

⁷ *J. Am. Pharm. Assoc.*, 10, 836 (1921).

carbon fraction of the oil is insoluble in 60 per cent acetic acid and that the ascaridole is miscible with this solvent; and in 1926 Paget¹ proposed a method, taking advantage of the oxidizing property of the organic peroxide ascaridole. It is with these two methods that this study is principally concerned.

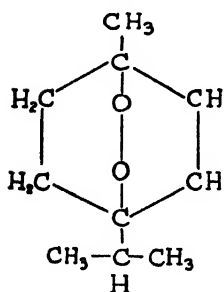
Until 1921 the United States Pharmacopeia stated that wormseed oil should be judged by its physical constants and specified that it have a specific gravity of 0.955–0.980 at 25°C. and an optical rotation of -4° to -10° , and that it be soluble in eight volumes of 70 per cent alcohol and have a refractive index of 1.4723–1.4770 at 20°C.

Nelson² pointed out that an estimate of the ascaridole in an oil, based on purely physical constants, was not reliable, since ascaridole is unstable under certain conditions and the oil may deteriorate without changing the physical constants. He then proposed the solubility method, details of which are as follows:

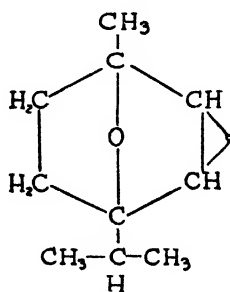
Ten cubic centimeters of chenopodium oil is agitated thoroughly in a cassia flask, the neck of which holds 10 cc. and is graduated in tenths, with 60 per cent acetic acid. The flask is then filled to the mark with 60 per cent acetic acid and allowed to stand or carefully centrifuged. The volume of the undissolved oil deducted from ten, multiplied by ten, gives the volume percentage of ascaridole in the sample.

This method was adopted by the U. S. P. as official.

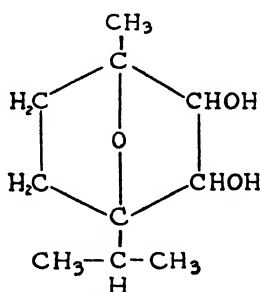
In his report on the use of the Nelson method, Paget³ pointed out the fact that ascaridole readily reverts to the inactive form, ascaridole glycol anhydride, Formula II, by an intramolecular change and that this product is readily hydrated to form ascaridole glycol (Formula III).



FORMULA I



FORMULA II



FORMULA III

Paget further stated that he found evidence of this change taking place by the application of either dry heat or steam. Since the oil is extracted commercially by steam distillation, it seems likely that such a change would occur. The anhydride and glycol are soluble in 60 per

¹ *Analyst*, 51, 170 (1925).

² *J. Am. Pharm. Assoc.*, 10, 836 (1921).

³ *Loc. cit.*

cent acetic acid and therefore would be determined as ascaridole by the Nelson method. Paget also referred to the adulteration of the oil with substances such as cineole, which is soluble in acetic acid.

Previous work by Wallach¹ demonstrated the possibility of the reduction of ascaridole with hydrogen in the presence of palladium. The reduction product was 1-4 terpin, corresponding to the addition of four atoms of hydrogen. Based on this fact, Paget sought to find a suitable reducing agent for the peroxide. He finally selected titanium trichloride (TiCl_3) as the most satisfactory. From the constitution of the peroxide, reduction should correspond to the addition of four atoms of hydrogen, but the amount used was about one-third of this; assuming the addition of but two hydrogens, the amount of TiCl_3 used was about three-fourths of the theoretical amount. Examination of the reduction products by this author did not show the presence of the anhydride or of the glycol. Hence, Paget was forced to rest the determination on the empirical factor that one gram of ascaridole is reduced by 1.2770 grams of TiCl_3 . This figure was the mean of a number of results varying from 1.240 to 1.3040. Details of the method as described by him are as follows:

Titanous chloride solution was prepared and standardized as described by Knecht and Hibbert², 66 cc. of the commercial 15 per cent solution being made up to 2250 cc. One gram of chenopodium oil was diluted with 96 per cent alcohol to 100 cc. and to 10 cc. of this solution in a flask, through which a current of carbon dioxide was passing, an excess of titanous chloride was added; the flask was then closed with a Bunsen valve, and its contents were heated almost to boiling for 1 or 2 minutes. If the pale violet color of the titanous chloride disappeared, more was added to insure the presence of an excess. The formation of a precipitate of titanic oxide during heating did not interfere with the determination. About 1 cc. of 5 per cent potassium thiocyanate solution was then added, and the solution was titrated back with a standard solution of iron alum until a permanent faint red color was obtained. The amount of iron used, calculated in terms of titanous chloride, gave by difference the quantity of the titanous chloride oxidized.

STUDY OF THE PAGET METHOD.

Since the determination of ascaridole by the Paget method is based upon an empirical factor, it was first essential to verify its value. A quantity of ascaridole, designated as oil A, was obtained, redistilled five times, and that portion giving the following constants: boiling point 85°C . at 5 mm., optical rotation = -2° , refractive index = 1.4745, and specific gravity 1.0029, was selected as a standard for the factor. This oil showed a value of 100 per cent ascaridole by the Nelson method, being completely soluble in 60 per cent acetic acid at 25°C .

In a preliminary study of the standardization of this method, it was noted that the concentration and volume of hydrochloric acid, added to the titanous chloride in preparing the standard solution as recommended

¹ *Ann.*, 60, 392 (1912).

² *New Reduction Method in Volumetric Analysis*, 1925.

by Knecht and Hibbert, affected the value of the empirical factor obtained. (This observation was noted by Knaffl-Lenz and Hofmann and reported by these authors, *loc. cit.*)

To test the effect of different concentrations of hydrochloric acid used in the titanium trichloride reagent, the following solutions were prepared: Solution No. 1 contained 22 cc. of hydrochloric acid per liter of TiCl_3 solution; solution No. 2 was prepared as per Paget's directions, 44 cc. of concentrated hydrochloric acid per liter; and solution No. 3 contained 88 cc. per liter volume. The acid employed was 36.5 per cent. These solutions were standardized against a 1 per cent alcoholic solution of the standard oil A. The determinations were carried out following Paget's directions. The time of heating was one and one-half minutes in each case. The results are given in Table 1.

TABLE 1.

Effect of hydrochloric acid on the standardization of TiCl_3 .

| NO. | HCl PER LITER | ACID NORMALITY OF SOLUTION | NORMALITY OF TiCl_3 | FACTOR (TiCl_3 OXIDIZED PER 1 GRAM OF ASCARIDOLE) |
|-----|------------------|-------------------------------|---------------------------------|--|
| | | | | grams |
| I | 22 | 0.284 | 0.032 | 1.295 |
| II | 44 | 0.568 | 0.033 | 1.283 |
| III | 88 | 1.136 | 0.034 | 1.186 |

The data in Table 1 show very clearly the influence of concentrated hydrochloric acid on the reduction of the peroxide, and the value of the factor for TiCl_3 . If it is assumed that two atoms of hydrogen are involved in the reduction of the compound, two molecules of titanous trichloride would be required; or for each gram of ascaridole 1.8378 grams of TiCl_3 would be necessary. Hydrochloric acid is necessary to stabilize the TiCl_3 reagent. However, it seems to change part of the ascaridole, whether by molecular rearrangement or by actual reduction, so that the empirical factor for TiCl_3 is dependent upon the concentration of hydrochloric acid used in preparing the reagent, as the above findings confirm.

Owing to the factor variation shown in Table 1, the preparation and standardization of the titanous chloride used in these studies were as follows:

Two hundred cubic centimeters of titanous chloride solution (15-20 per cent) was boiled with 400 cc. of concentrated hydrochloric acid (36.5 per cent) in a flask, cooled, and diluted to 9 liters. It was kept in a storage bottle, and the bottle was completely filled. The outlet of the bottle was fitted with a rubber stopper through which a piece of glass tubing was inserted. The tube was bent downwards, and a pinch cock

was placed at the rubber connection. Glass tubing formed the connection to the bottom of the buret. The stopper in the storage bottle had two holes, one leading to the top of the buret and the other to a bottle containing alkaline pyrogallol solution and from thence to a Kipp hydrogen generator. The apparatus was air-tight, and the pyrogallol solution was changed frequently to prevent oxidation of the TiCl_3 . With these precautions a solution of approximately 0.02 N TiCl_3 was obtained.

With the standard solution adjusted as described, samples of oil A, approximately 1 cc., were introduced by a pipet into a clean, dry, weighed 100 cc. volumetric flask; the flask and oil were reweighed, and the weight of the oil was recorded. The flask containing the oil was then filled to the mark with 96 per cent alcohol, and the total weight was taken. Next, a 10 cc. aliquot was removed, and the weight was obtained by difference. The aliquot was immediately placed in the titrating flask under carbon dioxide, and the procedure described by Paget was followed. Sixteen determinations were made upon samples of the standard oil A. The results are listed in Table 2.

TABLE 2.
Value of the TiCl_3 factor.

| SAMPLE NO. | TiCl_3 TO REDUCE ONE GRAM OF ASCARIDOLE |
|----------------|---|
| | <i>grams</i> |
| 1 | 1.288 |
| 2 | 1.290 |
| 3 | 1.287 |
| 4 | 1.285 |
| 5 | 1.305 |
| 6 | 1.294 |
| 7 | 1.281 |
| 8 | 1.298 |
| 9 | 1.264 |
| 10 | 1.271 |
| 11 | 1.277 |
| 12 | 1.292 |
| 13 | 1.284 |
| 14 | 1.275 |
| 15 | 1.282 |
| 16 | 1.277 |
| <hr/> | |
| Average Factor | 1.284 |
| Paget Factor | 1.277 |
| <hr/> | |
| Difference | 0.007 |

Table 2 gives the number of grams of titanous trichloride required to reduce one gram of the standard oil A used in establishing the value of the Paget factor. The minimum value was 1.264, the maximum 1.305. The average is 1.284, which checks within 0.007 of the Paget factor; the variation is doubtless due to the difference in the strength of the hydrochloric acid used by the author of the method and that used in preparing the solution for these studies.

COMPARISON OF NELSON AND PAGET METHODS.

The factor value in the Paget method having been established, attention was directed to the accuracy of the Nelson and Paget methods in determining the percentage of ascaridole under varying conditions of concentration. For this work a quantity of commercial oil was obtained from E. W. Pickett, a producer of Carroll County, Maryland. This oil, referred to later as oil B, had a gravity of 0.9840 at 25°C. It contained 88 per cent ascaridole by the Nelson solubility assay and 91.78 per cent by the Paget method. This oil was distilled under a pressure of 8–10 mm., and the following data were obtained:

| TEMPERATURE RANGE | OIL |
|-------------------|-----|
| °C. | cc. |
| 65–95 | 349 |
| 94–101 | 538 |
| Above 101 | 517 |

The fraction boiling between 65°–95°C., known to be largely cymene, is designated as oil C; the higher boiling fraction (above 101°C.), as oil D; and the middle fraction (94–101), known to be largely ascaridole, as oil E. In addition to these oils, oils F and G were obtained from plants grown at College Park, Maryland; H is a sample obtained from Baltimore, and I, J, and K were obtained from distillers in Carroll County, Maryland.

The percentage of ascaridole in these oils was determined by the two methods, data for which are listed in Table 3.

TABLE 3.
Ascaridole in commercial oils.

| | | NELSON ASSAY | PAGET ASSAY |
|-----------|---|-----------------|-----------------|
| | | <i>per cent</i> | <i>per cent</i> |
| Group I | A | 100 00 | 100 00 |
| | E | 100 00 | 100 00 |
| Group II | D | 98 00 | 93 19 |
| | F | 70 00 | 67 81 |
| | G | 70 00 | 66 81 |
| | H | 75 00 | 59 95 |
| | I | 70 00 | 68 95 |
| | K | 75 00 | 74 90 |
| Group III | B | 88 00 | 91 78 |
| | C | 35 00 | 45 49 |
| | J | 62 00 | 63 00 |

Oils A and E represent samples of ascaridole of the highest purity. Oil A was used to establish the value of the Paget factor. Oil E was prepared with the greatest care, and based on the factor 1.284 is 100 per

cent pure by the Paget method. Both of these oils were completely soluble in 60 per cent acetic acid, or gave 100 per cent purity by the Nelson method. The remaining oils in this list fall into two classes: Oils D, F, G, H, I, K (Group II) and B, C and J (Group III). Group II shows a higher percentage of ascaridole by the Nelson method than by the Paget assay, and the reverse is noted in Group III.

Paget pointed out that the Nelson method is based on the fact that the hydrocarbon fraction of chenopodium oil is insoluble in 60 per cent acetic acid and that the ascaridole is miscible with this reagent, but that ascaridole readily forms ascaridole glycol anhydride by an intramolecular change and that this product is readily hydrated to form ascaridole glycol, both of which are soluble in 60 per cent acetic acid. He further stated that he had found evidence of this change taking place by the application of either dry heat or steam.

Since the oils in Group II were obtained by the usual method of steam distillation of the herb, it is apparent that a fraction of the ascaridole would be converted into its tautomeric forms, ascaridole glycol anhydride and ascaridole glycol, and being soluble in 60 per cent acetic acid accounts for the high values by the Nelson method.

To confirm this theory further, portions of oils I, J and K were heated to 115°C. under vacuum and then allowed to stand in clear glass-stoppered bottles from February to May, when determinations were again made. Results of this experiment are given in Table 4.

TABLE 4.
Ascaridole as shown by Paget and Nelson methods.

| OILS | FEBRUARY | | MAY | |
|------|-----------------|-----------------|-----------------|-----------------|
| | Nelson | Paget | Nelson | Paget |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| I | 70 00 | 68 95 | 70 00 | 67 03 |
| J | 62.00 | 63.00 | 62 00 | 61 22 |
| K | 75 00 | 74 90 | 75 00 | 69 27 |

The slightly lower figures obtained by the Paget method indicate the deterioration of the oil due to intermolecular change, whereas the same values obtained by the Nelson method at the end of the experimental period showed no deterioration of the oil.

Oils B, C and J, or Group III, present a little different problem. Here a lower percentage of ascaridole is recorded by the Nelson method than by the Paget. Oil C is the first fraction obtained from the distillation of oil B; it consists largely of cymene. When the Nelson method is applied under such conditions, ascaridole and cymene, and ascaridole and 60 per cent acetic acid are found to be miscible with one another

in all proportions, but cymene and 60 per cent acetic acid are only partially miscible. If, therefore, to an oil containing cymene, as is the case with American wormseed oil, 60 per cent acetic acid is added, ascaridole distributes itself between the two liquid layers, and two conjugate ternary solutions, each consisting of ascaridole, cymene and acetic acid, are thereby produced. These two solutions are in equilibrium with each other, and the composition of each will depend upon the concentration of the three components and the temperature of the mixture. In the Nelson method, therefore, the cymene layer should always retain some ascaridole, and the amount would depend upon the concentration of the cymene in the oil. It is on this assumption that the low results obtained by the Nelson method are accounted for in oils B, C and J.

To verify the above assumption further, oil E, the purified middle fraction from B, was used in a series of dilution experiments with highly purified cymene, the principal constituent of the hydrocarbon fraction of chenopodium oil. Oil E was completely soluble in 60 per cent acetic acid and titrated 100 per cent ascaridole by the Paget method, when the factor 1.284 was used. The purest cymene available for these studies contained 10.95 per cent ascaridole by the Paget method and 7.00 per cent by the Nelson assay. This was redistilled until no ascaridole was detected by either method. Nine samples of these two oils were prepared by weighing each component, the ascaridole being varied from 10 to 90 per cent at approximately 10 per cent intervals. The results of this study are listed in Table 5.

TABLE 5.

Ascaridole in samples adulterated with cymene.

| SAMPLE NO. | AS MADE | NELSON ASSAY | ERROR— NELSON ASSAY | PAGET ASSAY | ERROR— PAGET ASSAY |
|---------------|-----------------|-----------------|------------------------|-----------------|-----------------------|
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1 | 9 86 | 9 00 | 8 70— | 9.85 | 0 10— |
| 2 | 19 98 | 18 00 | 9 99— | 19 99 | 0 05+ |
| 3 | 29 41 | 24 00 | 18 60— | 29 62 | 0 82+ |
| 4 | 39 71 | 30.00 | 24 45— | 39 26 | 1.10— |
| 5 | 49 88 | 38 00 | 23 90— | 50 55 | 1 34+ |
| 6 | 59 74 | 46 00 | 23 00— | 59 22 | 0 87— |
| 7 | 69 78 | 55 00 | 19 76— | 68 99 | 1 13+ |
| 8 | 79 58 | 70 00 | 12 10— | 79 63 | 0 09+ |
| 9 | 89 34 | 88 00 | 2 00— | 88 41 | 1.05— |

Consideration of the data in Table 5 shows the accuracy of the Paget method in measuring the active constituent in chenopodium oil. The maximum error recorded is within 2 per cent of the exact amount present. The percentage deviation by the Nelson assay is recorded as 2–24 per cent. Repeated determinations gave the same values. The separation in every determination was sharp, and the two layers were quite clear. The points of maximum and minimum deviation are shown more clearly in the graph.

The graph shows the Nelson method to be a three component extraction phenomenon. The appearance of two minima in the curve expressing the results is obvious, since 60 per cent acetic acid dissolved completely 100 per cent ascaridole, and when there is no ascaridole at all none can be lost; it follows that the error in the extraction must approach zero for both very high and very low percentages of ascaridole. The location of the maximum error and the slope of the error curve naturally depend on the slope of the isotherum for the ternary system in question.

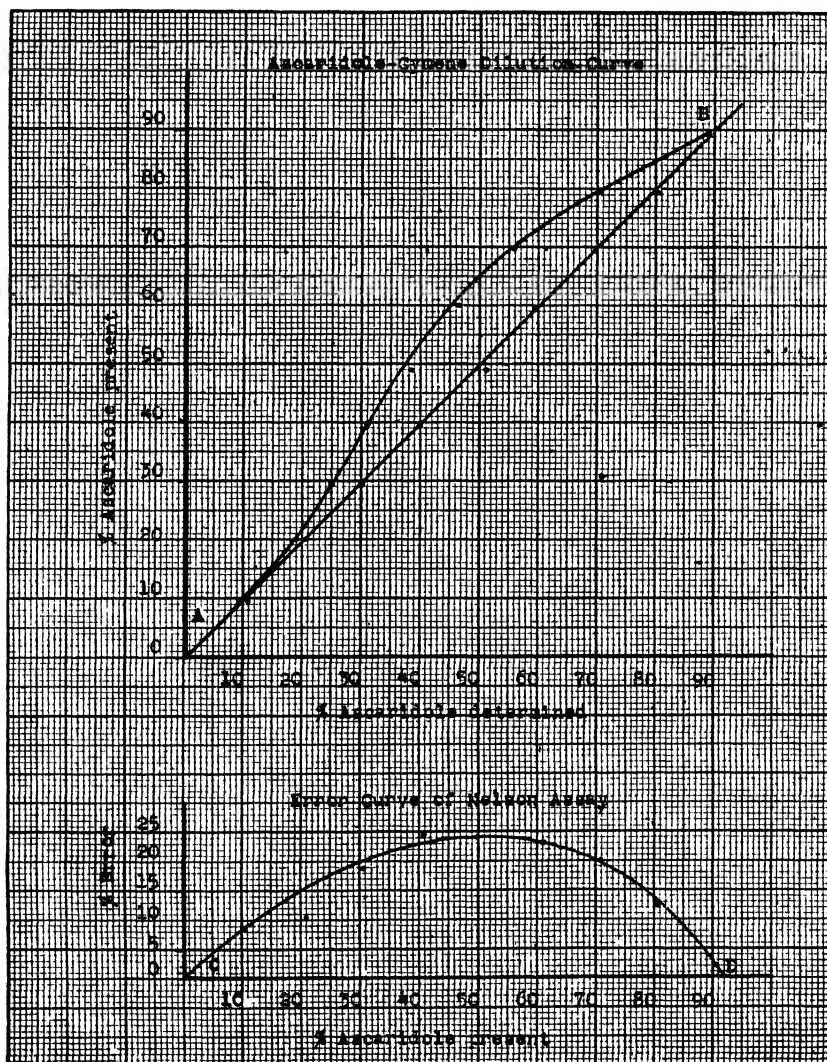


FIGURE 1.

SUMMARY.

An examination of the literature reveals that a number of qualitative and quantitative methods have been proposed for the estimation of ascaridole in oil of chenopodium.

Of the quantitative methods, the Nelson extraction and the Paget reduction methods have been studied. A third method, depending on the color reaction between hydrochloric acid and ascaridole, has been proposed by Knaffl-Lenz and Hofmann. Preliminary studies, not reported, indicate that it warrants consideration.

Studies confirming the factor proposed by Paget in the reduction method reveal that the exact value of the factor is dependent upon the concentration and quantity of hydrochloric acid used in preparing the titanous chloride solution.

The factor value obtained in these studies with highly purified ascaridole was 1.284, a value higher by 0.007 than that proposed by the author of the reduction method.

When the Nelson extraction method and the Paget reduction assay were compared, they gave identical values with two highly purified samples of ascaridole.

When these methods were used on fourteen other oils of varying composition, two sets of data were obtained. In the first, the Nelson method gave higher results than the Paget assay; in the second, the reverse was recorded. The high results obtained in the first group by the extraction method is accounted for by the presence in the oil of ascaridole glycol anhydride and ascaridole glycol. In the second group the low results by this same method are accounted for by the presence of *p*-cymene, the principal constituent of the hydrocarbon fraction of American wormseed oil. This is confirmed by a series of dilution studies with highly purified ascaridole and cymene.

These studies show that the Paget method is reliable under the conditions outlined, and that the Nelson method is not reliable as an indicator of the percentage of ascaridole contained in an oil. The Paget method differentiates between the ascaridole and its glycol and anhydride on the one hand, and is not influenced by the presence of the hydrocarbons in chenopodium oil on the other. Very small samples are required for its use, and it is accurate within the limits of experimental error allowable for analytical methods.

RECOMMENDATIONS¹.

It is recommended—

(1) That the titanium trichloride method be further studied collaboratively.

(2) That the Knaffl-Lenz and Hofmann colorimetric method be studied as a possible tentative assay.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 60 (1930).

REPORT ON SALICYLATES AND OTHER PHENOLS IN MIXTURES.

By F. C. SINTON¹ (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

The U. S. P. assay of phenol is really an estimation of total phenolic compounds; it does not differentiate between salicylic acid and phenol. Since the separation of these two compounds is frequently desired, the proposed method has been developed. This method depends on the use of sodium bicarbonate. The separation is carried out by shaking an ether solution of the salicylic acid and phenol with sodium bicarbonate solution. The sodium bicarbonate, by its limited alkalinity, neutralizes the salicylic acid and removes it from the ether, whereas the phenol is not removed. The associate referee conducted preliminary work in order to ascertain whether phenol was removed by the action of sodium bicarbonate. For this purpose an ether solution of phenol was treated with sodium bicarbonate solution, the amounts prescribed in the proposed method being used. Results showed that no phenol was removed.

COLLABORATIVE SAMPLE.

The sample submitted for collaborative study was a solution containing 4.95 grams of salicylic acid and 5.0 grams of phenol in 1000 cc. The exact quantity of phenol was added to the collaborative sample by means of a solution of absolute phenol in water. This solution was assayed by the U. S. P. method, and the volume containing 5.0 grams of phenol was added to the collaborative sample.

The salicylic acid was of U. S. P. grade and contained 99.5 per cent by U. S. P. assay; a sufficient quantity was added to make 4.95 grams of actual salicylic acid in the collaborative sample.

The method used is as follows:

REAGENTS.

- (a) *U. S. P. ether.*
- (b) *Dilute sulfuric acid (1 + 9).*
- (c) *Saturated sodium bicarbonate solution.*
- (d) *Chloroform-ether solvent.*—Mix 2 volumes of chloroform with 1 volume of ether.
- (e) *0.1 N sodium hydroxide.*
- (f) *Neutral alcohol.*
- (g) *Dilute sodium hydroxide solution.*—4 grams to 100 cc.
- (h) *Potassium iodide solution.*—1 gram in 5 cc. of water, freshly prepared.
- (i) *0.1 N sodium thiosulfate.*
- (j) *0.1 N bromine solution.*

PREPARATION OF SAMPLE.

Powders.—Weigh into a volumetric flask a sufficient quantity of the powder so that an aliquot of 25–50 cc. will contain approximately 0.13 gram of phenol. If acid, make

¹ Presented by H. Runkel.

alkaline with dilute sodium hydroxide and add 25 cc. in excess; fill to mark with water; and shake well.

Liquids.—For liquid samples proceed as directed under determination.

DETERMINATION.

(a) *Salicylic Acid*.

Transfer to a separatory funnel a sufficient quantity of solution to represent about 0.13 gram of phenol. Acidify with dilute sulfuric acid and extract with ether, using 20, 15, 15 and 10 cc. portions, respectively, and continue the extraction with the solvent until complete. Combine the ether in a separatory funnel, and shake with sodium bicarbonate solution, using 15, 15 and 10 cc. portions, respectively, and finally 15 cc. of water. Combine the sodium bicarbonate solutions and extract with 15 cc. of ether. Add the latter to the main bulk of ether and reserve for the phenol determination. Acidify the sodium bicarbonate solution with strong hydrochloric acid. Extract with chloroform-ether solvent, using 30, 25, 20 and 10 cc., respectively, until the salicylic acid is completely removed. Filter the solvent into a beaker through cotton previously saturated with chloroform. Evaporate to 5 cc. on a covered steam bath with the aid of an electric fan. Allow the last 5 cc. to evaporate spontaneously. Dissolve the residue in 10 cc. of neutral alcohol and titrate with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator.

Each cc. of 0.1 *N* sodium hydroxide = 0.01381 gram of salicylic acid ($C_6H_4OHCOOH$).

(b) *Phenol*.

Shake the reserved ether solution with two 15 cc. portions of dilute sodium hydroxide, then with 15 cc. of water. Transfer the extractions to a 100 cc. volumetric flask and fill to mark. Transfer an aliquot representing 0.03–0.04 gram of phenol to a glass-stoppered Erlenmeyer flask, add 30 cc. of bromide-bromate solution, cool the flask, and add 7 cc. of concentrated hydrochloric acid. Close the flask immediately. Shake during one-half hour, remove the stopper just sufficiently to introduce quickly 5 cc. of an aqueous solution of potassium iodide, being careful that no bromine vapor escapes, and immediately stopper the flask. Allow to stand 15 minutes, remove the stopper, wash with distilled water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate.

Each cc. of 0.1 *N* bromine = 0.001568 gram of phenol (C_6H_5OH).

Results reported are as follows:

| | SALICYLIC ACID | PHENOL |
|--|---------------------|---------------------|
| | gram per 100 cc. | gram per 100 cc. |
| E. O. Eaton | 0 496 | 0.499 |
| U. S. Food, Drug and Insecticide Adm. San Francisco, Calif. | 0 488 | 0.50 |
| Earl L. Anderson | 0 481 | 0 508 |
| U. S. Food, Drug and Insecticide Adm. Baltimore, Md. | 0.483 | 0.508 |
| M. Harris | 0.492 | 0.491 |
| U. S. Food, Drug and Insecticide Adm. Chicago, Ill. | 0.49 | 0.49 |
| F. C. Sinton | 0 493 | 0.498 |
| | 0 494 | 0.502 |
| | 0.490 | 0.497 |

COMMENTS BY COLLABORATORS.

E. O. Eaton.—I have no comments to make other than that the method seems satisfactory.

Earl L. Anderson.—The method was followed in all details, except in the salicylic acid determination the chloroform-ether solvent was washed with 5 cc. of water to avoid possible carrying over of hydrochloric acid.

M. Harris.—The method is very accurate and needs no comment. The only suggestion I can offer is that it may be advisable to wash the chloroform-ether extract of the salicylic acid with water and filter the solvent through a small dry filter, thus assuring a thoroughly dry and acid-free solvent.

DISCUSSION.

The usual precaution of washing the chloroform-ether solvent containing the extracted salicylic acid was omitted from the method sent to the collaborators. This was noted in comments by Anderson and Harris. As a whole, the results obtained by the collaborators are satisfactory, the total average recovery of salicylic acid being 98.9 per cent and that of phenol 99.8 per cent.

It is recommended that the method be adopted as tentative¹.

REPORT ON SMALL QUANTITIES OF IODIDES IN MIXTURES.

By T. F. PAPPE (U. S. Food, Drug and Insecticide Administration, Baltimore, Md.), *Associate Referee*.

For the determination of small quantities of iodides in mixtures, two methods were selected for study. The first method was the well-known iodate method, depending upon direct titration of the iodide with potassium iodate in the presence of chloroform, and the second was the A. O. A. C. method used for the determination of iodine in brines. These methods were adapted to drug conditions as follows:

METHOD I.

REAGENTS.

Potassium iodate.—5.3505 grams of KIO_3 per liter. (KIO_3 is considered a stable salt. It is best, however, to standardize this solution by adding 2 grams of KI and 5 cc. of concentrated HCl to a measured portion and titrating with 0.1 *N* thiosulfate. 1 cc. of 0.1 *N* $\text{Na}_2\text{S}_2\text{O}_3$ = 0.003567 gram of KIO_3 .)

Concentrated hydrochloric acid.

Sodium hydroxide.—*Sodium carbonate solution.*—2.5 grams of each per 100 cc. *Chloroform.*

REACTION.



¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 66 (1930).

DETERMINATION.

Take a sufficient quantity of the sample to represent 0.1–0.2 gram of KI. If solid, moisten with water and add sufficient sodium hydroxide-sodium carbonate solution to make distinctly alkaline. Dry, and char thoroughly below dull redness. Dissolve the ash in hot water, filter into a 250 cc. glass-stoppered Erlenmeyer flask, wash well, and adjust to 20 cc. volume. Add 35 cc. of concentrated hydrochloric acid and 5 cc. of chloroform and titrate with the iodate solution. The color of the solution due to liberated iodine will increase and then gradually decrease as the titration proceeds. The end point is reached when the pink color of the iodine just disappears from the chloroform layer after vigorous shaking. Report results as percentage potassium iodide, if solid, and grams per 100 cc. if liquid.

1 cc. of KIO_3 = 0.008301 gram of KI, 0.007496 gram of NaI, and 0.006346 gram of I.

METHOD II.

REAGENTS.

(a) *Sodium hydroxide-sodium carbonate solution.*—Dissolve 50 grams of a mixture of equal weights of sodium hydroxide and sodium carbonate in water and dilute to 1 liter.

(b) *Dilute sulfuric acid.*—Dilute 10 cc. of concentrated sulfuric acid to 100 cc.

(c) *Sodium hydroxide solution.*—Dissolve 4 grams of sodium hydroxide in water and dilute to 100 cc.

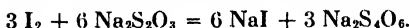
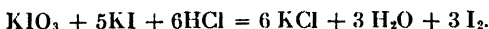
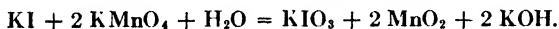
(d) *Potassium permanganate solution.*—Dissolve 100 grams of potassium permanganate in water and dilute to 1 liter.

(e) *95 per cent alcohol by volume.*

(f) *Potassium iodide crystals.*

(g) *Standard sodium thiosulfate solution.*—Dissolve 12.4 grams of recrystallized sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in water and dilute to 1 liter.

REACTIONS.



DETERMINATION.

Take such a quantity of the sample as will contain not more than 0.1 gram of potassium iodide. If solid, moisten with water, and add sufficient of the sodium hydroxide-sodium carbonate solution to make distinctly alkaline. Dry, and char thoroughly below dull redness. Dissolve the ash in water, filter, and wash well with hot water. Introduce the filtrate into an Erlenmeyer flask, adjust the volume to about 100 cc., neutralize with the dilute sulfuric acid, and add 1 cc. of the sodium hydroxide solution. Heat to boiling; add an excess of the potassium permanganate, about 0.5 cc.; continue heating until the precipitate begins to coagulate; and allow to cool. Add sufficient 95 per cent alcohol or hydrogen peroxide to bleach the permanganate color, and set the beaker on a steam bath. When the precipitate has settled, filter and wash with hot water. After cooling, add 1–2 grams of potassium iodide, acidify with strong hydrochloric acid, and titrate with the standard thiosulfate solution. One-sixth of the iodine titrated represents the quantity originally present. 1 cc. of 0.05 *N* sodium thiosulfate solution is equivalent to 0.001384 gram of KI, 0.001249 gram of NaI, and 0.001058 gram of I.

Both methods gave good results on ordinary inorganic mixtures. The presence of chlorides in reasonable amounts and of metals, with the exception of mercury and arsenic, was not found to interfere. Accordingly, a quantity of elixir of sodium salicylate compound, N. F. V, was carefully prepared, but only one-half the formulary requirement of potassium iodide was used. It was proposed to furnish this material to collaborators for further study of the two methods. Upon examination of the material, however, there was found a serious shortage in iodide content. This was eventually traced to loss of iodide in the course of settling and filtration of the elixir, the iodide being carried down in part with the resinous material and removed therewith during filtration. By the time this difficulty had been discovered, it was too late to expect collaborative work for this season. It is recommended¹, therefore, that these methods be again subjected to collaborative study. It is also recommended that further work be done for the purpose of making a method general in scope, particularly insofar as interference of mercury and arsenic is concerned.

REPORT ON BISMUTH COMPOUNDS IN TABLETS.

By J. CALLOWAY, JR. (U. S. Food, Drug and Insecticide Administration, New York, N. Y.), *Associate Referee*.

Work on bismuth compounds in tablets was undertaken for the first time this year. No limitations were set as to the type of these tablets, so the work was begun with the idea of finding a method that would be applicable to any bismuth compound in any tablet. It was soon found, however, that no such method was available.

Two general types of bismuth compounds are found in tablets: the inorganic, of which bismuth subnitrate and bismuth subcarbonate are probably the most common, and the organic, of which bismuth subgallate is an example.

In many organic combinations bismuth is not precipitated completely by the usual reagents, unless the compound is so treated that the organic portion is destroyed. This destruction is rendered difficult by the fact that the usual wet combustion method, with a mixture of nitric and sulfuric acids, leaves the bismuth in an almost insoluble form. A method of combustion proposed by the Contact Committee of the American Drug Manufacturers' Association was tried. It is rather dangerous to use, however, because the oxidation of tablets containing beta-naphthol and similar compounds by nitric acid may yield explosive products.

A method of combustion using sodium peroxide was tried and seemed to be promising. This work was not carried far enough to justify recom-

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 66 (1929).

mending its use, however. These difficulties are not encountered in dealing with inorganic compounds of bismuth, and it was therefore decided to restrict the first work to tablets containing such compounds only.

Since any tablets ordinarily will contain either starch or talc or both in addition to the medicinal agents present, a method was sought which would not be affected by these binders or by other inorganic medicinal ingredients which might accompany the bismuth compound.

The Contact Committee has proposed a method of solution in nitric acid, after charring by heating with this acid. Two methods of precipitation were proposed—one with ammonium carbonate in alkaline solution, the other with ammonium phosphate in acid solution. Both of these have disadvantages. An alkaline precipitation in a solution from tablets of unknown composition seemed unsatisfactory. The precipitation of bismuth from an acid solution as a phosphate is probably more characteristic for bismuth, but the possibilities of inaccurate results due to variations in the acidity of the solution and the physical character of the precipitate are greater.

The possibility of a volumetric method was considered, but owing to the large atomic weight of bismuth, with the resultant lack of sensitivity of volumetric solutions, it was considered undesirable to use a volumetric method.

Since the method of phosphate precipitation from an acid solution seemed the most promising, three samples for collaborative work by this method were sent out. These samples were of the following composition: (1) Bismuth subnitrate, U. S. P.; (2) ground tablets of bismuth subnitrate and magnesium oxide; and (3) ground tablets of bismuth subnitrate, cerium oxalate and cocaine hydrochloride.

These were sent out with the following directions (practically the identical method of the Contact Committee):

PREPARATION OF SOLUTION.

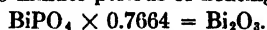
Weigh 5 grams of powder into a 500 cc. Kjeldahl flask and add 30 cc. of water. Shake the mixture and add about 20 cc. of concentrated nitric acid in small portions, with agitation. Heat the mixture gradually until all the organic matter has been destroyed, adding more nitric acid if necessary. Cool the solution, transfer to a 500 cc. glass-stoppered flask (filtering if not clear), using 1 per cent nitric acid for washing. Dilute the contents of the flask to the mark with 1 per cent nitric acid. If a precipitate forms, add sufficient dilute nitric acid to dissolve it before making up to the mark.

Precipitation as BiPO₄

Transfer 50 cc. of the solution containing bismuth to a 300 cc. beaker and add ammonia water in small portions through a dropping funnel, stirring after each addition until a slight permanent precipitate is obtained.

Add 4 cc. of concentrated nitric acid, heat to boiling, and add a solution consisting of 1 gram of monobasic ammonium phosphate, 2 cc. of concentrated nitric acid, and 48 cc. of water. Boil for 5 minutes and allow to stand on the steam bath for several

hours, replacing water lost by evaporation. Collect the precipitate in a weighed Gooch crucible (dried to constant weight in a muffle at low red heat) and wash with hot water. Dry the crucible and contents at 100°C. and heat to constant weight in a muffle. Two 15 minute periods of heating at dull red heat should be sufficient.



NOTE: (1) BiPO_4 is hygroscopic and this fact must be kept in mind in weighing the dried salt. (2) The ammonium phosphate solution may be obtained by neutralizing 12 cc. of 85 per cent phosphoric acid with ammonia water (about 15 cc. of concentrated NH_4OH), using methyl orange as indicator, and making up to 1000 cc. with the addition of 40 cc. of concentrated nitric acid. 50 cc. of this solution contains approximately 1 gram of monobasic ammonium phosphate.

The collaborators were requested to make calculations in terms of bismuth oxide, thus temporarily avoiding the difficulty of deciding the factor for converting bismuth phosphate to bismuth subnitrate, the formula for which is indefinite.

Collaborators reported their results as tabulated below (per cent Bi_2O_3 in each case):

| | 1 | 2 | 3 |
|-----------------------------------|-----------|-----------|-----------|
| S. Reznek—New York Station | 77.22 | 31.21 | 31.12 |
| F. D. and I. Administration | 76.48 | 31.31 | 31.10 |
| | 77.10 | 31.34 | 31.14 |
| | Av. 76.93 | Av. 31.29 | Av. 31.12 |
| T. N. Bennett—New York Station | 79.65 | 30.88 | 32.26 |
| F. D. and I. Administration | 79.80 | 30.78 | 32.10 |
| | 80.06 | 31.04 | |
| | Av. 79.84 | Av. 30.90 | Av. 32.18 |
| E. O. Eaton—San Francisco Station | 78.75 | 31.3 | 31.4 |
| F. D. and I. Administration | | | |
| W. S. Hubbard—Baltimore Station | 75.44 | 29.28 | 31.03 |
| F. D. and I. Administration | 74.95 | 29.43 | 31.35 |
| | 74.80 | | |
| | Av. 75.06 | Av. 29.35 | Av. 31.19 |
| Maurice Harris—Chicago Station | 79.05 | 31.34 | 32.50 |
| F. D. and I. Administration | 79.10 | 31.26 | 32.50 |
| | 79.35 | 31.46 | 32.74 |
| | Av. 79.17 | Av. 31.35 | Av. 32.58 |

COMMENTS.

The following comments were made:

W. S. Hubbard.—The presence of oxalates, gallates, and such organic compounds makes it exceedingly difficult to obtain complete oxidation by the method given, and the subsequent solution of the bismuth. The method has been tried on bismuth subgallate without success. It would seem better to treat the powder several times with HNO_3 , dry, and ignite gently.

Maurice Harris.—No difficulty was encountered with the directions. The only question in my mind regarding the method is whether it is necessary to heat the bis-

moth phosphate on the steam bath for several hours, since heating for one hour seemed to settle the precipitate, at the same time assuring a sufficiently dilute nitric acid solution for the complete retention of the precipitate.

It will be noted that on sample No. 1, which was U. S. P. bismuth subnitrate, results reported were not satisfactory. A U. S. P. assay of this product gave 79.34 per cent Bi_2O_3 . This may not represent exactly the content of Bi_2O_3 , but results by phosphate precipitation tend to be low.

In sample No. 2 the bismuth content as bismuth oxide, figured from the manufacturer's formula, and assuming the bismuth subnitrate used yielded 79 per cent bismuth oxide, was 31.81 per cent. Besides binders, the tablets from which this sample was prepared contained magnesium oxide. The results, though probably a little low, are fairly satisfactory and apparently there is no interference by magnesium.

In sample No. 3, calculated as above described, the content of bismuth as bismuth oxide was 29.30 per cent. This sample contained, in addition to the binders used, cerium oxalate and a small amount (about 2 per cent) of cocaine hydrochloride. The proportion of cerium oxalate was the same as that of bismuth subnitrate. The results on this sample are somewhat higher than the theoretical, and this is probably due to contamination from the cerium present.

In view of the facts presented, the associate referee does not think that this method is suitable for adoption by the association at this time and therefore recommends that further work be done toward the development of a better and more comprehensive method¹.

REPORT ON COLORIMETRIC METHODS FOR VITAMINS.

By E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.),
Associate Referee.

No experimental studies of color reactions for the evaluation of foods and drugs with respect to vitamin potency were made by the associate referee during the year, but a review of the literature was prepared. It summarizes in a fairly comprehensive way the experiences and suggestions of various workers in this field. The evidence at present does not justify the conclusion that chemical tests thus far devised can be relied upon as adequate measures of vitamin potency, but some of the procedures suggested appear to be promising. Spectrometric methods supplemented by color tests are regarded by qualified critics as worthy of further investigation.

A review of the literature, with a bibliography appended, appears in the following paper:

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 66 (1930).

VITAMIN COLOR REACTIONS—A REVIEW OF THE LITERATURE.

By H. J. FISHER and E. M. BAILEY (Connecticut Agricultural Experiment Station, New Haven, Conn.).

The laborious, expensive and time-consuming character of biological assays for the vitamins, as well as their limitations in distinguishing small quantitative differences, have led a number of investigators to seek for some chemical means of evaluating the vitamin content of food materials.

This review is an attempt to show the present state of knowledge of colorimetric methods for estimating vitamins, in which class belong all the chemical methods thus far used. Each of the four vitamins for which color tests have been proposed will be discussed in order.

VITAMIN A.

The Drummond-Watson Test.

The first attempt at developing a colorimetric test for vitamin A was made by Drummond and Watson (17)¹, who observed a parallelism between the intensity of the well-known color given by cod-liver oil and sulfuric acid and the potency of the oil in feeding experiments. They dissolved the oil in petroleum ether and determined the dilution at which a purple color was just visible. The reaction was found to be given by the livers of many animals, as well as by butter; it was given by the unsaponifiable fraction even when decolorized by "Norit". Destruction of vitamin A by aeration ran parallel with diminution of the intensity of the color reaction.

The Rosenheim-Drummond Test.

Unfortunately, the purple color given by cod-liver oil and sulfuric acid disappears rather quickly and is likely to be masked by charring. Attempting to find other and more satisfactory reagents, Rosenheim and Drummond (55) discovered several compounds that gave a blue color with cod-liver oil: arsenic trichloride, dimethyl sulfate, trichloroacetic acid, and, in the presence of zinc chloride, acetyl and benzoyl chlorides. Of these, arsenic trichloride proved the most satisfactory. One cc. added to 1 drop oil gave a blue color which was permanent enough to be measured, and parallelism was observed between the intensity of the color and the potency of the oil. Cholesterol gave no reaction until heated, and then only a permanent red color. The authors originally compared their oils against a standard dye solution made from crystal

¹ See bibliography.

violet and methylene blue, but in the method as finally worked out they compared the colors in a Lovibond tintometer, using the same technic as that given in the Carr and Price test to be next described. Incidentally it may be stated that Carr and Price (12) found that carefully purified trichloroacetic acid, contrary to the claims of Rosenheim and Drummond, did not give a blue color with cod-liver oil, but that traces of phosgene or dimethyl sulfate caused the reagent to become effective.

As Kahlenberg (39) had earlier noted that impure varieties of cholesterol gave a blue color with arsenic trichloride, the arsenic trichloride test has sometimes been called the Kahlenberg test, although Kahlenberg made no claims that the color was a measure of vitamin A.

The Carr-Price Test.

The next step was the introduction by Carr and Price (12) of the use of antimony trichloride. They objected to arsenic trichloride for two reasons: because it was poisonous in character, and because the reaction would not work without a great decrease of sensitivity if either the reagent or the oil were diluted with an inert solvent. They found that when 2 cc. of a saturated solution of antimony trichloride in chloroform was treated with 0.2 cc. of a 20 per cent solution of cod-liver oil, also in chloroform, a fairly stable blue color was obtained, which gradually faded to a red and finally a red-brown. This test has been more thoroughly investigated than any other, and conditions for its use have been carefully studied. It has been found that the reaction is affected by traces of moisture and alcohol, and by temperature, and its intensity by the concentration of antimony trichloride and by the presence of impurities in the chloroform (chlorine, phosgene) (13, 38, 70, 75, 81, 82, 83). With very careful drying Wokes (80) even succeeded in getting colors stable for nearly an hour, although ordinarily the color begins to fade after one minute; Towle and Merrill obtained colors stable for one hour on working at 2°C. (69).

The method as finally worked out consists in treating 0.2 cc. of a 20 per cent chloroform solution of the oil with 2 cc. of a nearly saturated solution of antimony trichloride in chloroform, and immediately (within 30 seconds) comparing the color with Lovibond blue glasses in a tintometer. The temperature should be 15°-16°C. If the color reading is over 10 Lovibond units, a more dilute solution of the oil should be taken. For details as to the technic of the test the papers of Wokes and Willmott (82, 83) and of Drummond and Baker (14) should be consulted. A special colorimeter for facilitating comparisons is described by Rosenheim and Schuster (56).

In this connection it should be noted, however, that in a personal communication of Hawk (33) to the writers he criticizes the use of the

30 second reading and claims that the maximum blue color, irrespective of time and temperature, should be taken; some of his experiments with dilute antimony trichloride solutions point towards the maximum being reached in less than 30 seconds with certain oils under the usual conditions of the test.

Various attempts have been made to use standard colored solutions instead of the Lovibond glasses (3, 37, 78), but those workers who have had most success with the method have not found this satisfactory, owing to the fact that the color may contain varying proportions of red and yellow as well as blue.

In considering the reliability of the antimony trichloride test as a measure of vitamin A, it is necessary to distinguish between the test as applied to cod and other liver oils, and as applied to other materials. On the first point there is much more evidence than on the second. Most workers in this field have found a very close parallelism between the results of the color test and those of feeding experiments, and have found that treatments such as irradiation and aeration, which destroy the vitamin, similarly affect the color reaction. Drummond and Baker (14) even go so far as to say, "We * * * believe it (the antimony trichloride test) to be a more accurate method of estimating vitamin A than the biological method". Coöperative experiments by the Accessory Food Factors Committee for the League of Nations Health Organization (1, 42) led to a qualified approval of the test.

It has been generally held that the arsenic trichloride and antimony trichloride tests give identical results (1).

On the other hand, Euler, Euler and Karrer (23), Karrer, Euler and Euler (40), and Euler and Euler (20) have found that carotin, the bixin of annatto, crocetin of saffron, lycopin from tomatoes—in fact, all carotinoids, and even some starches—give a blue color with antimony trichloride. This fact is of less importance in the case of cod-liver oil than that of other foodstuffs. Even with other foodstuffs it might not be important if these authors had not raised again the old question as to whether carotin can have the physiological properties associated with vitamin A (18, 23, 48, 63). Some of the compounds used by Euler and Euler, although giving a deep blue with antimony trichloride, did not possess any curative properties, and they concluded that the reaction was a general one for "polyenes", some sort of molecular compound being formed. In fact, they succeeded in isolating a compound of carotin and antimony trichloride in crystalline form (27).

Sexton (61) quotes Windaus, Borgeaud and Brunken as saying that ergosterol peroxide and ergopinacone, although biologically inactive, give a blue color with antimony trichloride. Steudel (65) found that some substances which were proved to contain vitamin A by feeding experiments reacted negatively to the antimony trichloride reagent, and

Steudel and Peiser (66), Jones, Briod, Arzoomanian and Christiansen (38), and Bailey, Cannon and Fisher (3) failed to find the intensity of the color reaction always proportional to the vitamin A potency.

The most damaging evidence against the Carr and Price test is afforded by the experiments of Hawk (32, 33). He found that exposure of certain samples of cod-liver oil to the air caused a very definite increase in the intensity of the blue color, and states that, "We have * * * failed absolutely to find any correlation between the colorimetric and the biological methods for the determination of vitamin A" (33). The observation of an increase in blue color on exposure to air he has had corroborated from another source. If these experiments cannot be questioned, it would seem impossible to place any reliance on the Carr and Price test, except possibly as a sorting test to see what oils it might be worth while to assay biologically.

If the experience of Hawk could be disregarded, and if the somewhat doubtful evidence that carotin may be one form of vitamin A were ignored, it might still be possible with suitable safeguards to develop the Carr and Price test into a reasonably satisfactory method of estimating vitamin A. It has been shown by several writers that the carotinoids can be adsorbed from ether solutions with charcoal, without the charcoal apparently taking up any vitamin (17, 64, 76). The combined artifices of saponification, ether extraction and decolorization with charcoal would probably remove most interfering substances and enable the test to be applied to other foodstuffs than cod-liver oil. In fact, Andersen and Nightingale (2) did apply such a method to the examination of such diverse materials as oleo oils, cakes, bread and eggs, and found their results consistent with biological tests. R  si   (60), Wokes and Willimott (84) and Kerppola (41) likewise used the test with varied materials; the paper of the last writer unfortunately is in a rather inaccessible journal.

There is still another way in which the blue given by vitamin A (assuming that it is vitamin A that gives the blue color) might be distinguished from that caused by other substances. Wokes (80) in some careful experiments with a spectrograph found a sharply defined absorption band at about $614\ \mu\mu$, while the blue color given by carotin shows a band at $590\ \mu\mu$ and none in the region of $610\ \mu\mu$ (49). While somewhat outside the scope of this article, it may be noted that Morton and Heilbron (51) have found that (in the untreated material) an absorption band at $320\ \mu\mu$ appears to accompany vitamin A in a wide range of substances, while the carotinoids give no marked bands in the ultra-violet (48).

The best opinion as to the value of the Carr and Price test, at least until recently, may be summed up in the words of Moore: "For the present it seems safe to assume that materials which give no blue color-

tion with antimony trichloride even after removal of saponifiable matter must be devoid of vitamin A activity. Materials of liver oil origin giving colour reactions characterized by absorption at $610\text{ }\mu\mu$ may be considered active. But, on the other hand, materials which give colour reactions characterized by absorption at other positions may be either active or inactive, or mixtures of active and inactive chromogens, and the biological technique still remains the only satisfactory method of assay" (49).

The Fearon Test.

Fearon originally proposed two tests (28). One may be merely mentioned and then disregarded as it has not been further investigated, and is scarcely adaptable to quantitative use: about 1 gram of phosphorus pentoxide is added to 5 cc. of oil or of a solution of oil in petroleum ether, and the mixture is shaken. A deep violet color develops slowly, changing to a muddy brown. The test may also be made by letting a drop of oil fall on some solid phosphorus pentoxide.

In Fearon's second test in its original form, one or two drops of oil or of an ether solution of the substance to be tested were treated with a little solid pyrogallol and 5 cc. of a 12 per cent solution of trichloroacetic acid in petroleum ether. A bluish pink color slowly developed, changing to deep rose and reaching its maximum intensity in 15-20 minutes. Fearon stated that the rate of development of the color might be hastened by warming or by adding a little benzoyl peroxide.

Willmott and Moore (73) investigated and modified this test. Their final quantitative procedure was as follows: To 2-8 drops of oil, 8 per cent ethereal resorcinol was added in an amount equal to 0.5 per cent of the oil, then 5 cc. of 10 per cent trichloroacetic acid in toluene and 0.5 cc. of a saturated solution of benzoyl peroxide in the same solvent. After 2 hours the color was compared against a solution of magenta toned with methylene blue.

Other work (75) on this test may be disregarded since the negative value of the test is shown by the investigation of Rosenheim and Webster (57, 59). Briefly, these authors found that the unsaponifiable fraction of cod-liver oil did not give the Fearon reaction, while it was given by the unsaturated fatty acids of the oil. Pig's liver fat, although highly potent biologically, gave no color with the reagent, while sardine oil, which had no growth-producing properties whatever, gave an intense color reaction. They believed the chromogen was an aldehydic oxidation product of a highly unsaturated fatty acid of the type of clupanodonic acid.

Other Tests.

The numerous other color tests for vitamin A proposed by various authors have apparently not been studied independently by other in-

investigators. Some of them are not well adapted to quantitative application. They will be merely described here without comment.

Carr and Price (12), besides their antimony trichloride reagent, described several other tests:

- (1) Stannic chloride in chloroform gives a deep blue rapidly changing to purple.
- (2) Anhydrous ferric chloride added to a solution of cod-liver oil in chloroform gives a fluorescent reddish violet color.
- (3) Anhydrous aluminum chloride added as a powder to an oil gives a red-violet fading to brown.
- (4) If an oil is dissolved in chloroform containing phosgene or dry hydrogen chloride and a trace of aluminum chloride is added, a purple color is produced. Without phosgene the color is red and fades quickly.
- (5) Silicon tetrachloride added to cod-liver oil gives a rose-pink color; the reaction is not given by cholesterol.
- (6) Phosphorus oxychloride gives a transient blue rapidly fading to red.

Wilson (78) found that ether extracts of liver treated with fuming nitric acid gave very deep purple to bluish pink colors, which faded rapidly. The color was roughly proportional to the intensity of the antimony trichloride reaction.

Kerppola (41) found that sulfuric acid and phosphorus pentoxide together gave a blue-violet color with substances containing vitamin A. He apparently also rediscovered the benzoyl chloride-zinc chloride reaction of Rosenheim and Drummond (see page 352), although he obtained a rose rather than a blue color.

Kobayashi and Yamamoto (43) state that Japanese acid clays, Florida earths and fullers' earth give bluish-green sediments with cod-liver oil and vitamin A concentrates from other sources. The sample in benzene solution is treated with the clay. They claim to have worked out a quantitative method of applying this test. They also state that anhydrous zinc chloride, aluminum chloride, phosphorus pentoxide and sulfuric acid give the same reaction.

Hamano (30), besides mentioning a number of the previously described tests and adding magnesium and titanium chlorides to the lists of substances giving a blue color with vitamin A, describes the following color reactions:

On adding to an alcoholic solution of "biosterol", a vitamin concentrate from cod-liver oil, the reagents enumerated below plus a few drops of concentrated hydrochloric acid, the following colors were observed:

- (1) Phloroglucinol: green turning blue, remaining unchanged 10 minutes, then turning Prussian blue and finally rose-pink. In the case of cod-liver oil itself, the color is pink, then green and then very slowly blue.
 - (2) Orcinol: lemon-yellow.
 - (3) Pyrogallol or resorcinol: green for a few minutes.
 - (4) Naphthol-resorcinol: a deep green persisting several days.
 - (5) Aniline, xyridine, benzidine, naphthylamine or phenylhydrazine: crimson red.
- In this case glacial acetic acid is also added.

- (6) Indole: dark green gradually turning brown.
- (7) α -Methyl indole: blue as in (1).
- (8) Skatole: yellowish brown.
- (9) Pyrrole: green changing immediately to dark brown.
- (10) Quinaldine: yellowish brown.

Hamano found that the color reactions with phloroglucinol and naphthylamine were effective with a concentration of $\frac{1}{600,000}$ of "biosterol". Many aldehydes when condensing with one or other of the above reagents give colors or precipitates, and furfural shows a close resemblance to "biosterol" in its color reactions. However, "biosterol" also reduces Fehling's solution, ammoniacal silver oxide and phosphomolybdic acid. The above color reactions are not given by cholesterol.

VITAMIN B.

The first suggestions for a color test for the estimation of vitamin B were that the Folin-Macallum and Folin-Denis reagents might be employed (19, 72). These suggestions have never been carried further, however. The only color test for vitamin B seriously investigated is that of Jendrassik (36).

The Jendrassik reagent is made by freshly mixing equal volumes of 0.1 *N* ferric chloride and potassium ferrocyanide. A concentrated aqueous solution of the preparation to be examined containing 2 per cent of acetic acid is treated with the reagent until the maximum depth of the resulting blue color is attained. The mixture is allowed to stand 10 minutes, and the color is observed; then 1-5 volumes of water are added and the color is again observed. If there is a distinct blue color, and on standing a bright blue precipitate, the test is considered positive. Jendrassik states that some vitamin-free extracts give a green color, but never a blue. On prolonged boiling with 5 per cent sodium hydroxide, vitamin-containing products lose their power to give the blue color, and give only green.

The first criticism of the Jendrassik reaction was made by Bezssonoff (7, 11), who pointed out that the same reaction was given by ortho and para phenols. Levine (46, 47) independently discovered that the Jendrassik test was given by a great many phenols; glucosides such as arbutin, esculin and salicin that yield phenols on hydrolysis; phenolic alkaloids such as morphine, codeine, apomorphine and cotarnine; thiourea, uric acid, para-formaldehyde, *p*-dimethylaminobenzaldehyde, Michler's ketone, sodium taurocholate, hydroxylamine hydrochloride, benzidine, toluidine, *p*-phenylenediamine hydrochloride, and alpha and beta-naphthylamine. All these compounds also reacted negatively after boiling with sodium hydroxide. He also pointed out that the test had been recommended by Hager in 1873 as a test for morphine.

VITAMIN C.

There is likewise only one color reaction that has been proposed for the detection and estimation of vitamin C. Bezssonoff (4, 6) discovered that a certain phosphomolybdotungstic acid gave a blue color with plant juices containing vitamin C, as well as with hydroquinone, catechol and pyrocatechol. He later modified the reagent by the isolation and purification of the complex acid in crystalline form, and believed he had worked out the conditions under which the test could be applied quantitatively for the estimation of vitamin C (8, 9, 10). He believed that it was not vitamin C itself which gave the reaction, but a decomposition product of it.

Various workers have criticized the Bezssonoff test (44, 54, 71), but the most conclusive examination was that of Glassmann and Posdeew (29). They worked out very careful quantitative methods of applying the test, only to find that the reaction was given by tannins at ordinary temperature, and by carbohydrates on heating. Uric acid also reduced the reagent. Glassmann and Posdeew found the color developed with the materials examined was proportional to their tannin and carbohydrate content, and decided that the test was valueless.

VITAMIN D.

The Shear Test.

Shear (62) proposed the following color test for vitamin D:

Three cubic centimeters of a mixture of 1 part of concentrated hydrochloric acid and 15 parts of aniline is mixed with an equal volume of cod-liver oil and boiled half a minute. A green color and then a red is formed. The emulsion separates on standing into two layers, the lower of which is an intense red which deepens on standing.

Rosenheim and Webster (85) corroborated Shear's statement that ordinary irradiated cholesterol gave the test, but found that antirachitic cholesterol that had been irradiated in an atmosphere of nitrogen did not. Air-irradiated specimens which had lost their antirachitic properties still gave the aniline reaction. They found that inactive oils after the addition of a trace of benzoyl peroxide or turpentine gave the reaction, and suggested that it was probably due to organic peroxides. Sexton (61) also found that Shear's test was given by materials containing no vitamin D.

The Bezssonoff Test.

Bezssonoff (5) noted that when a one-third solution of cod-liver oil in benzene was shaken with a few drops of his vitamin C reagent, the benzene layer on separating was colored orange yellow. He believed the color was due to a derivative of vitamin D. Rosenheim and Webster (85) dispute this, and Sexton (61) says that the reaction is not given at all by irradiated ergosterol.

The Stoeltzner Test.

Stoeltzner (67) has lately suggested a test for vitamin D which is really the same as the first test proposed by Fearon for vitamin A. All that is known about this test may be stated in quoting Stoeltzner's very short article verbatim:

"Setzt man dem Vigantöl, das eine 1 proz. Lösung von antirachitischem Vitamin in Olivenöl ist, Phosphorperoxyd zu, so sieht man alsbald eine von dem zugesetzten Phosphorperoxyd ausgehende rötlichbraune Färbung auftreten, die allmählich immer dunkler, zuletzt fast schwarz wird.

"Gewöhnliches Olivenöl ohne oder mit Zusatz von nicht gestrahltem Cholesterin gibt diese Reaktion nicht; ebenso wenig Lipanin oder Sesamöl. Wohl aber gibt Lebertran die gleiche Reaktion.

"Es handelt sich also wohl um eine chemische Reaktion zwischen Phosphorperoxyd und antirachitischem Vitamin".

It should be stated that while nearly all users of the Carr and Price test have assumed that it was given only by vitamin A, and Willimott and Wokes (76) found that a vitamin D concentrate gave a negative test, Nelson in a private communication has suggested that both vitamins A and D may give the reaction.

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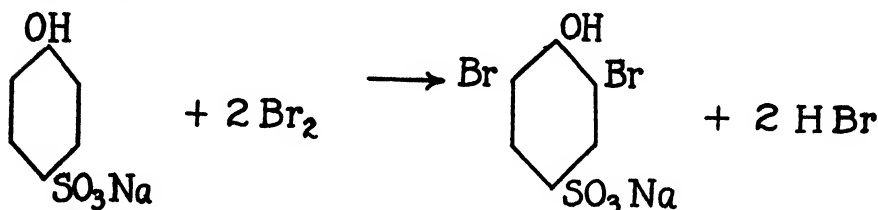
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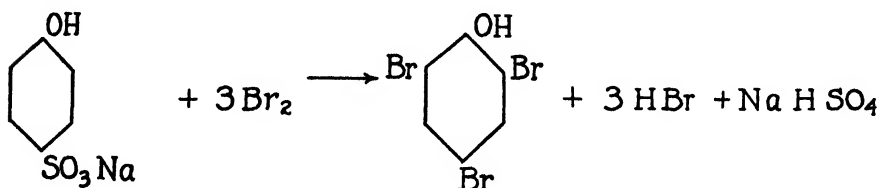
REPORT ON PHENOLSULFONATES.

By M. HARRIS (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

The salts of phenolsulfonic acids have long been used as astringents, but they are not included in the 10th Revision of the U. S. Pharmacopeia. On account of their common use it seems desirable to have an official method for their assay. The assay of zinc phenolsulfonate described in U. S. P. IX is based on the determination of zinc as zinc sulfide, rather than on the active ingredient, sulfonic acid, and for this reason this method was not considered satisfactory for further study. The U. S. P. IX also includes an assay for sodium phenolsulfonate that depends on the formation of dibromophenolsulfonic acid, the reaction proceeding as follows:



In the course of preliminary work the associate referee confirmed that this method was not entirely satisfactory owing to the possibility of formation by a side reaction of tribromophenol, as was shown by a precipitate or turbidity after longer contact with bromine. The reaction proceeds as follows in the presence of water:



According to Andanti¹, in the presence of an acid the SO₃ group is liberated, which permits the reaction to proceed to complete conversion to tribromophenol. This action is the basis for the following proposed method:

REAGENTS.

- (a) 0.1 N bromide-bromate solution.
- (b) 0.1 N sodium thiosulfate solution.
- (c) Concentrated sulfuric acid.
- (d) 10 per cent potassium iodide solution.
- (e) Starch indicator.

¹ *Boll. chim. farm.*, 56, 317-318 (1917).

PREPARATION OF SAMPLE.

Weigh accurately a suitable quantity corresponding to 0.4–0.5 gram of the phenol-sulfonate. Place in a 500 cc. volumetric flask and make up to volume.

Method I.

Transfer a 50 cc. aliquot into a 250 cc. glass-stoppered flask and add 30 cc. of the bromide-bromate solution. Shake the mixture and cool to about 10°C. Add 10 cc. of concentrated sulfuric acid, immediately insert the stopper, and after shaking again let stand in the dark at room temperature for 3 hours.

Remove the stopper just sufficiently to introduce quickly 10 cc. of fresh 10 per cent potassium iodide solution, being careful that no bromine vapor escapes, and at once stopper the flask. Shake and let stand 1 hour. Titrate the iodine liberated with the 0.1 *N* sodium thiosulfate solution, using starch indicator. 1 cc. of 0.1 *N* bromide-bromate = 0.00463 gram of zinc phenolsulfonate, $\text{Zn}(\text{C}_6\text{H}_4\text{OH}.\text{SO}_3)_2.8\text{H}_2\text{O}$, or 0.00387 gram of sodium phenolsulfonate, $\text{NaC}_6\text{H}_4\text{OH}.\text{SO}_3.2\text{H}_2\text{O}$.

Method II.

Transfer a 50 cc. aliquot into a 500 cc. glass-stoppered flask and add 30 cc. of the bromide-bromate solution and 70 cc. of water. Shake the mixture and cool to about 10°C. Add 15 cc. of concentrated sulfuric acid, immediately insert the stopper and, after shaking again, let stand in the dark at room temperature for 2 hours. Proceed as in Method I, beginning with "Remove the stopper * * *".

Prior to submitting the material for collaborative study, samples of zinc and sodium phenolsulfonate, labelled U. S. P. IX, were assayed by the proposed methods. The results obtained, ranging from 99.0 to 99.8 per cent, justified their use for further collaboration.

COLLABORATIVE SAMPLE.

Two samples, consisting of equal parts of zinc phenolsulfonate and lactose and sodium phenolsulfonate and lactose, were submitted to the collaborators. The results obtained by the associate referee are as follows:

| | METHOD I | METHOD II |
|------------------------|-----------------|-----------------|
| | <i>per cent</i> | <i>per cent</i> |
| Zinc phenolsulfonate | 49.5 49.8 | 49.4 48.9 |
| Sodium phenolsulfonate | 49.5 49.1 | 49.2 49.5 |

The results of examination by E. H. Grant of the Baltimore Station are as follows:

| | METHOD I | METHOD II |
|--|-----------------|-----------------|
| | <i>per cent</i> | <i>per cent</i> |
| Zinc phenolsulfonate + 8H ₂ O | 50.0 49.23 | 49.03 48.94 |
| Sodium phenolsulfonate + 2H ₂ O | 45.94 45.47 | 48.68 48.22 |

It is suggested by Grant that the following modification be tried:

Mix the 70 cc. of water with the 15 cc. sulfuric acid, cool to 25°C., and add to the mixture of 50 cc. of sample and 30 cc. of bromate. This ought to obviate the necessity of cooling the mixture to 10° and eliminate any danger of decomposition of the bromide by the concentrated acid with the liberation of bromine. This extra bromine would make the results low.

Since the results of three other collaborators were not received, it is recommended that further study be made on the assay of salts of phenol-sulfonic acid¹.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 66 (1930).

CONTRIBUTED PAPERS.

ANALYTICAL SUBLIMATION WITH SPECIAL REFERENCE TO THE FIELD OF MICROSUBLIMATION.

By HENRY HOFFMANN, JR., and W. C. JOHNSON (State Agriculture,
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INTRODUCTION.

Sublimation and its application in the various branches of science have only in recent years assumed a major part in the field of chemistry. Although extensive work has been done in the past, the erroneous information and figures in reference books and tables and the lack of standard apparatus have retarded its progress.

In order to illustrate the wide divergence of results found by different workers and the lack of knowledge as to what constitutes sublimation, a few figures are quoted. One reference work states that benzoic acid sublimates at 100°C., and another gives 140°C. Since benzoic acid melts at 120°–124°C., it is obviously impossible for it to sublime at 140°C. Cantharadin is said by one authority to sublime at 82°C. and another author states that it sublimates above the melting point. Cinnamic acid is said not to sublime, and pyrogallol (M. P. 128–134°C.) is said to sublime at 210°C. *vacuo*.

It is true that the sublimation point is not a fixed point as is the boiling point or the freezing point, but with a standard apparatus the sublimation point could be affixed within certain definite limits. Sublimation depends upon the vapor pressure of the substance, but in order to observe the process the distance through which the subliming particles have to travel in order to be deposited on the surface must be taken into account. Thus, two operators observing the same substance might obtain different results if one tried to sublime the substance through a distance twice as great as the other. This phenomenon is not true of all substances, but it does apply to a few. For instance, in the case of indigo the sublimate appears to be driven up only a few millimeters, no matter how much the temperature is increased, and the observer sees a sort of blue haze just above the solid indigo. Since, in the experiments made by the writers, the slide was placed an inch above the substance to be sublimed, this action of indigo was not classed as sublimation. Kempf¹ has the deposit surface in his sublimation cell placed only 0.1–0.01 mm. above the substance to be sublimed, and hence he obtained a sublimate of indigo. However, with the surface of deposit so close to the substance

¹ *Z. anal. Chem.*, 62, 284–93 (1923).

to be sublimed, there seems to be a question as to whether the action he observed is true sublimation or some other mechanical phenomenon.

Quantitative and qualitative analyses appear to afford fields for the practical application of sublimation and a great number of experiments have been made to determine the speed of sublimation. In a majority of cases the compounds examined have been found to sublime quantitatively in a surprisingly short period of time. For example, the writers have found that in the determination of saccharin it is both quicker and more accurate to dispense with the customary fusions and to sublime it from the dried ether extract for both quantitative and qualitative determinations. It has also been found to be a great aid in the determination of vanillin and coumarin in the analysis of vanilla extract, while its application in the analysis of drugs seems to be but a question of time.

To insure the successful sublimation of a compound it is often necessary in the case of a gummy or sirupy mixture to purify the material to some extent. Some difficulty is also encountered when an attempt is made to separate by means of sublimation two or more substances that have low melting points that are quite close together. It is well known that in a mixture of two or more organic compounds the melting points of both substances are lowered, as each compound acts as an impurity to the other. Thus, when an attempt is made to separate vanillin and coumarin by sublimation the two compounds melt at a low temperature and sublimation of either is impossible.

Most of the early investigators used an apparatus of a very simple design for sublimation under partial vacuum as well as under atmospheric pressure. The watch-glass and the tube forms were followed by the retort type of sublimator, and later the bell jar type was adopted. Kolbe's device consisted of a pair of watch-glasses ground to fit, and held together by means of a clip. Liebig passed the inert gas, carbon dioxide, through a retort to facilitate the operation and prevent decomposition. The tube-type sublimators, which found favor among a large number of early investigators, consisted of two test tubes which fitted snugly one within the other. The bottom of the smaller tube was cut off, so that when it slid into the larger one it furnished a wall on which the sublimate deposited.

In 1911 Ray and Datta¹ designed a special apparatus for sublimation in partial vacuum. It consisted of a bent tube, the lower portion of which contained the substance to be sublimed; the upper vertical portion received the sublimate, and it was bent so as to prevent the sublimate from falling back into the lower portion. In 1912 Morey² designed an apparatus of the bell jar type for subliming naphthalene and benzoic acid in large quantities under partial vacuum. In 1925 Hedley³ designed

¹ *Proc. Chem. Soc.*, 27, 236 (1911).

² *J. Am. Chem. Soc.*, 34, 550-552 (1912).

³ *J. Chem. Ind.*, 44, 752 (1925).

an apparatus for sublimation under reduced pressure. This apparatus permits the removal of the sublimate intact or of the constituents of a sublimable mixture in successive stages during operation. The progress and temperature observations of the sublimation may be determined with accuracy.

Helwig¹ was one of the first to employ microsublimation systematically for the examination and recognition of alkaloids. His method was to place a small quantity of material in a depression on platinum foil, cover it with a slip of glass and then apply heat carefully from a small flame. In 1875 Guy² improved the process by using a "sublimation cell", consisting of a glass ring covered by a glass disc and supported by another disc. The cell was placed on a brass plate supplied with a thermometer and then heat was very gradually applied from a small flame midway between the cell and the thermometer.

The later work of Von Eder³ was carried out under reduced pressure with more carefully controlled methods of heating. Previous to this time microsublimation under vacuum had not been tried. He classified a number of alkaloids according to the observations made and pointed out that a large number of them, which formerly were not found to be sublimable, were rendered sublimable in vacuum.

In 1923 Kempf⁴ designed an apparatus that provided for working in vacuum or in inert gases. The apparatus, which consists essentially of an electric hot plate that is mounted on a porcelain base, is connected with a rheostat for temperature regulation. The substance to be sublimed is distributed in a thin layer on the hot plate, and the sublimate is collected on the microscopic slide, which is placed only about 0.10 to 0.01 mm. above the surface of the material.

A question may arise as to whether the action observed is true sublimation or a mere mechanical movement of particles since the distance of sublimation is only 0.10–0.01 mm.

HORTVET SUBLIMATOR.

The late Julius Hortvet designed a very satisfactory sublimator, which has been described in detail of construction and operation⁵. This sublimator and the atmospheric pressure sublimator were used in obtaining all results which are given in this paper. The Hortvet sublimator is adaptable to quantitative, qualitative and microsublimation under reduced pressure. The results at atmospheric pressure were obtained by means of another specially designed apparatus, consisting of an electric hot plate on which is placed a metal block which contains a cell and a

¹ *Das Mikroskop in der Toxicologie*.

² *Pharm. J. Trans.* (2), viij, 719; *Forensic Medicine*, London, ix, 10, 58 (1875).

³ *Schweiz. Wochschr.*, 51, 228, 241, 253 (1913).

⁴ *Anal. Chem.*, 1923, 62, 284–93 (1923).

⁵ *This Journal*, 6, 481 (1923); 8, 559 (1925).

thermometer. The cell is covered with a microscopic slide, which is cooled by a tube containing running water. A glass jacket resting on the hot plate entirely encloses the metal block which contains the cell and the thermometer. A cork in the upper end of this jacket contains openings through which the thermometer and cooling tube project.

Two Hortvet sublimators can be connected to the same vacuum pump and the same cooling system so as to insure like conditions in both instruments when they are used to obtain comparative results. When the apparatus is used for separations, purifications, or any quantitative analysis where large sublimates are received, they are collected on the inner bulb, but when a qualitative analysis is the object a microscope slide is placed over the dish and the sublimate is collected on this slide in a manner similar to that described for microsublimation under atmospheric pressure.

Whether microsublimation was done in vacuo or under atmospheric pressure with this apparatus, the distance through which the sublimate was passed was one inch. In order to secure uniform results it is necessary to observe this distance and keep it constant.

When a substance is to be sublimed it is placed into the small dish. This dish, together with the special thermometer, is placed in the aluminum receiver, and these parts are placed into the base of the sublimator.

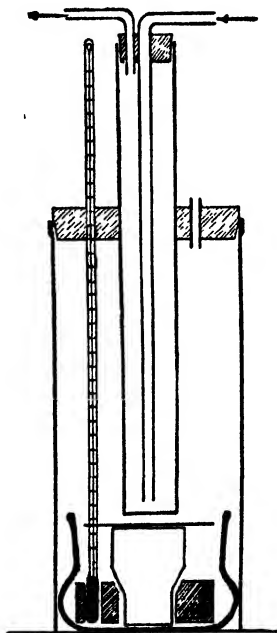


FIG. 1.—ATMOSPHERIC PRESSURE SUBLIMATOR.

The apparatus is then fitted together, the sublimator is placed into its special container, cold water is circulated through the inner bulb, the sublimator is connected to the vacuum pump, and the pressure is reduced to about 0.1 mm., the pressure being measured with a manometer. A thermometer is also provided in the container so that the investigator has two controls for taking the temperature, one outside and one inside the sublimator. Heat is applied at the base by means of a small Bunsen burner, and the temperature is raised to the proper point.

The Hortvet apparatus, so arranged, may be used for all purifications, separations, and other work encountered in quantitative analysis, the sublimate in these experiments being collected on the bulb. However, if qualitative analysis is desired, a microscope slide is placed over the sublimation dish, and the sublimate is collected on the slide and examined.

Figure 1 illustrates the modified apparatus employed by the writers in obtaining the sublimation results under atmospheric pressure. Two of these instruments may be operated at a time on the same electric hot plate so that comparative results may be obtained.

The apparatus is heated until a visible sublimate is obtained. Then the substance being sublimed is cooled at 2° intervals, the temperature being kept constant at each interval for 20 minutes; the last temperature at which a sublimate visible under the microscope was obtained was called the "sublimation point". The figures given in the table indicate the many compounds that may be sublimed under atmospheric pressure and demonstrate the applicability of atmospheric pressure sublimation.

EXPERIMENTAL DATA.

This article does not claim to give a complete list of sublimable compounds, but a sufficient number of substances have been examined so that it has been conclusively demonstrated that sublimable substances are to be found in all classes of compounds. Thus it has been found that some elements sublime, iodine and sulfur being examples. The inorganic division contains a large number of sublimable compounds, of which arsenious oxide and mercuric iodide will serve as illustrations. It remains, however, for the organic field to supply the great majority of compounds that exhibit the property of sublimation. In this division compounds of all classes are found to sublime and in the list of sublimable compounds are included those with chain structure and those with ring structure. The ring structures are of the carbocyclic and heterocyclic type, and in these great divisions may be found alcohols, amines, phenols, acids, alkaloids, and others. The great variety of these sublimable compounds may best be illustrated by Table 1, which gives the compounds and their "sublimation points".

TABLE 1.

Data on "sublimation points" under atmospheric pressure and in *vacuo* and also the temperatures and time required for quantitative sublimations. The table further includes the melting points and other misleading information regarding the "sublimation temperatures" of these compounds found in the literature.

| COMPOUNDS | °C. | MELTING POINTS AND DATA, COMPILED FROM REFERENCE WORKS °C. | OBSERVED SUBLIMATION TEMPERATURES— <i>in vacuo</i> Atmospheric pressure °C. | QUANTITATIVE SUBLIMATIONS 0.10 GRAM SAMPLES VACUUM: 0.50-1.0 mm. | | |
|---------------------|---------|--|--|---|----------------|----------------------|
| | | | | Tempera- ture °C. | Time hours. | Recovery per cent |
| Acetanilide | 112-116 | | 56-58 | 70 | 2.25 | 99.8 |
| dl-Alanine | 295 | | 135-137 | 180 | 2.25 | 99.6 |
| Alizarin | 282-299 | Sublimes at 110-140° above m. p. | 71-73 | 180 | 9.00 | 99.7 |
| Anthracene | 213-218 | Sublimes about m. p. | 77-79 | 100 | 5.00 | 99.1 |
| Anthranilic Acid | 143-145 | | 52-54 | 90 | 4.00 | 99.6 |
| Anthraquinone | 276-285 | Purified by sublimation | 26-29 | 140 | 3.50 | 99.6 |
| Arsenious Oxide | 200-275 | 193, 200, 218 | 92-95 | 150 | 1.00 | 99.7 |
| Aspirin | 132-136 | | 77-80 | 105 | 21.00 | 99.7 |
| Atropine | 97-118 | 93-110 (10 mm.) faint mist—123 | No sub. | 95 | 42.00 | 99.2 |
| Benzenehexachloride | 150-157 | Room temp., 100, 140, slightly above m. p. | 43-45 | 100 | 1.00 | 98.2 |
| Benzoic Acid | 120-124 | | Room temp. | 80 | 0.75 | 99.9 |
| d-Borncol | 203-208 | Does not sublime | Room temp. | 80 | 0.25 | 80.9 |
| Caffeine-anhydrous | 230-237 | 79, 180, sublimes 237, 135 — (100-3 mm.) | 72-74 | 150 | 0.50 | 99.9 |
| Cantharidin | 210-218 | 82-85, above m. p.—100-120 (partial vacuum) | 63-65 | 125 | 0.50 | 99.8 |
| Catechol | 103-105 | Can be sublimed | 36-38 | 75 | 0.50 | 100.0 |
| Chloranil | 283-290 | Sublimes readily | 63-65 | 105 | 3.00 | 99.6 |
| Cholesterol | 145-149 | 200-300 (in <i>vacuo</i>) | No sub. | 130 | 7.00 | 99.5 |
| Cinchonidine | 202-210 | | 167-170 | 170 | 2.25 | 97.5 |
| Cinchonine | 250-264 | Sublimes readily at 220, above m. p. | 82-86 | (200) | 0.75 | (99.7) |
| Cinnamic Acid | 132-133 | Does not sublime | 58-60 | 170 | 19.25 | 99.6 |
| Cocaine | 93-98 | 75-90 (10 mm.), 98, above m. p. | 52-56 | 90 | 2.25 | 99.7 |
| Codeine-anhydrous | 152-156 | 100-130 (10 mm.) | 48-51 | 85 | 35.00 | 99.6 |
| Colchicine | 142-147 | | 80-83 | 140 | 1.75 | 99.3 |
| Coumarin | 67-68 | | 92-96 | { In 6 hours sublimes 0.0053 g. at 135°C. On this basis would require 120 hours | | |
| Furoic Acid | 126-132 | | 30-33 | | | |
| | | | 41-43 | 75 | 1.00 | 97.9 |

| Hexamethylenetetramine | 280-281 | 230-250, about 263 | 45-47 | Room temp. | { In 7 hours sublimates 0.0023 g. at 125°C. On this basis would require 290 hours | 99.3 |
|------------------------|-------------|---|------------|------------|---|-------------|
| Hydrastine | 131-133 | | No sub. | 96-100 | | |
| Iodoform | 119-120 | Sublimes | 43-45 | 30-34 | 0 50 | 96 7 |
| Isatin | 200-201 | | 78-80 | 47-50 | 110 | 99 7 |
| Mercuric Chloride | 265-287 | Above m. p. | 48-50 | 37-40 | 0 50 | 99 7 |
| Mercuric Iodide | 238-241 | About 237 | 36-38 | 28-30 | 130 | 98 6 |
| Mercurous Chloride | 265-302 | 150, about 300 | 98-101 | 86-89 | 160 | 99 3 |
| Morphine-anhydrous | 230 (d) 254 | 150, clouds disc., 188 crystals, above m. p. | 188-191 | 56-60 | 200 | 99 3 |
| Naphthalene | 79-80 | Sublimes easily | 36-38 | Room temp. | 50 | 86.2 |
| b-Naphthol | 120-123 | Purified by sublimation | 43-45 | 33-35 | 75 | 99.6 |
| Oxalic Acid-anhydrous | 187-189 | 110 (10 mm.), 150-160 | Room temp. | Room temp. | 100 | 0.25 94.5 |
| Paraformaldehyde | 120-152 | | 75-77 | 59-63 | 100 | 7 00 1.6 |
| Phenolphthalein | 250-261 | | 230-235 | 129-134 | 220 | 1.25 99 5 |
| Phloroglucinol | 217-219 | Sublimes without decomposition | 107-110 | 75-79 | 150 | 6 50 100 0 |
| Phthalic Anhydride | 128-131 | Can be sublimed | 50-52 | 27-30 | 80 | 2 50 99 5 |
| Phthalimide | 228-231 | | 53-55 | 32-35 | 110 | 1.25 99 7 |
| Pyrogallol | 128-134 | | 47-49 | 32-35 | 80 | 2 00 98 7 |
| Pyromucic Acid | 131-134 | 210 (in vacuo), above m. p. | 50-52 | 29-32 | 85 | 0 50 99 4 |
| Quinine-anhydrous | 173-177 | 130-140 (50-60 mm.) | 157-160 | 99-103 | 165 | 1 00 99 3 |
| Quinidine-anhydrous | 168-171 | 130-148 (10 mm.) | No sub. | 92-96 | 150 | 12 00 99 4 |
| Quinone | 111-116 | Sublimes at room temp. | Room temp. | Room temp. | 65 | 0 50 70 2 |
| Resorcinol | 106-119 | Sublimes | 48-50 | 31-34 | 90 | 1 00 100 0 |
| Saccharin | 219 (d) 238 | 100, above m. p. | 84-86 | 59-63 | 150 | 1 50 99 9 |
| Saligenin | 82-86 | 100 - | 46-48 | 31-34 | 60 | 1 75 99 6 |
| Salicylic Acid | 155-159 | 75-76 (in vacuo), 200 - | 57-59 | 30-33 | 100 | 0 50 99 8 |
| Santonin | 165-171 | Above m. p., 155-160 (20-3 mm.) | 104-107 | 59-63 | 120 | 5 50 99 7 |
| Strychnine | 221-284 | 169, above m. p. | 190-192 | 150-154 | 225 | 1 50 99 8 |
| Succinic Acid | 150-190 | Sublimes as anhydride above b. p. | 59-61 | 48-51 | 165 | 1 25 98 6 |
| | | | | | (130 | 11 75 99 8) |
| Theobromine | 329-350 | 134, crystals at 170, sublimates without melting at 290-295 | 146-149 | 110-114 | 210 | 0 50 99 8 |
| Urea | 130-133 | 120-130 (in vacuo) | 59-61 | 49-52 | 95 | 3 25 99 2 |
| dl-Valine | 298 (d) | | 138-141 | 73-76 | 160 | 1 75 99 7 |
| Vanillin | 79-82 | Sublimes at 280 | 47-49 | 33-35 | 55 | 4 00 99 5 |
| Veronal | 182-191 | | 66-68 | 43-46 | 115 | 3 25 99 6 |
| Dimethylgluxime | 246 | | 43-45 | 36-39 | 140 | 0 25 99 4 |
| Benzidine | 122-128 | Capable of sublimation | 70-72 | 40-49 | 100 | 3 50 100 0 |
| Skatole | 95 | Decomposes on being heated | Room temp. | Room temp. | 75 | 0 25 95 5 |
| Hippuric Acid | 187 5-189 | | 43-45 | 38-41 | 160 | 0 50 99 5 |

As may be seen from the data given in Table 1, all the "sublimation points" are within a range of 5° or less. All these figures were obtained by means of microsublimation, and in all cases the microscopic slide was exposed for at least 20 minutes in order to obtain the lowest temperature at which a compound would sublime. In order that the reader may become acquainted with processes described in this paper, a brief explanation will be given. The substances were placed in the narrow dish, which was inserted in the aluminum receiver, the receiver carrying the thermometer. Over the dish bearing the substances to be sublimed was placed a clean microscope slide. The dish was then slowly heated until a visible sublimate was obtained. After it had been shown that the substance sublimed, sublimate was obtained at decreasing temperatures (temperature lowered 2°C. for each period) until the lowest temperature at which a sublimate is visible under the microscope was obtained. These last observations were taken over a period of 20 minutes, this time being decided upon as being a sufficiently long one for practical application in the laboratory. Fresh microscope slides were inserted at 2° intervals in order to fix the sublimation point as accurately as possible.

QUANTITATIVE AND QUALITATIVE SUBLIMATION.

In many operations in quantitative and qualitative analysis extractions are made with various solvents, the solvent is evaporated, and the residue is weighed or titrated to calculate its percentage; if the object be qualitative analysis, the residue may be subjected to other separations and tests. In many cases if sublimation were employed at this stage the results could be obtained with greater speed and accuracy. In quantitative analysis there may be many cases where the purity of the extracted substance may be questioned, and if the substance is merely weighed or titrated, errors might occur. For example, in the determination of benzoic acid in catsups, fruit juices and similar products, if the residue is subjected to sublimation instead of direct weighing and titration, and the sublimate is washed into a dish and weighed, the results obtained are more nearly correct.

If the object had been a qualitative test for benzoic acid, microsublimation could have been used to advantage since benzoic acid gives very characteristic crystals.

The writers made a toxicological examination of the stomach and contents of a dog. The usual qualitative tests indicated strychnine, and it was positively identified by producing the characteristic crystals of strychnine by sublimation. Sublimation may frequently be employed to separate two sublimable substances, for example caffeine and theobromine, since their sublimation points are widely separated. Saccharin in foods may be determined by sublimation. If the ether extract of the

TABLE 2.
Comparative results obtained in the determination of benzoic acid in fruit products.

| | MIRION ORANGE LAB. NO. 18 | PROPRATE, ORANGE FLAVOR LAB. NO. 7 | ORANGE CUP LAB. NO. 269 | APRICOT CONDIAL LAB. NO. 14 | GRAPE FRUIT GROUP LAB. NO. 16 | MANGOLIA STRIP LAB. NO. 147 | STRAWBERRY LAB. NO. 223 | APPLE CIDER LAB. NO. 1 | APPLE JUICE (BLANK) | APPLE JUICE† (NO. 1) | APPLE JUICE‡ (NO. 2) |
|--|------------------------------|--|----------------------------|--------------------------------|-------------------------------------|--------------------------------|----------------------------|---------------------------|------------------------|-------------------------|-------------------------|
| 1. Weight of crude chloroform extract (gram) | | 0.0134 | | 0.0154 | 0.0098 | 0.0134 | 0.0245 | 0.0107 | 0.0090 | 0.0448 | 0.0651 |
| 2. Loss in weight of dish and contents during sublimation (gram) | | 0.0120 | | 0.0131 | 0.0074 | 0.0110 | 0.0229 | 0.0086 | 0.0032 | 0.0355 | 0.0608 |
| 3. Hence, percentage of benzoic acid | | 0.0800 | | 0.0436 | 0.0246 | 0.0366 | 0.0763 | 0.0570 | 0.0106 | 0.1183 | 0.2026 |
| " " sodium benzoate | | 0.0944 | | 0.0514 | 0.0290 | 0.0432 | 0.0900 | 0.0673 | 0.0125 | 0.1396 | 0.2391 |
| 4. Weight of sublimate (gram) | 0.0141 | 0.0098 | | 0.0127 | 0.0052 | 0.0100 | 0.0213 | 0.0086 | 0.0032 | 0.0351 | 0.0604 |
| 5. Hence, percentage of benzoic acid | 0.0470 | 0.0660 | 0.0533 | 0.0423 | 0.0173 | 0.0333 | 0.0710 | 0.0570 | 0.0106 | 0.1170 | 0.2013 |
| " " sodium benzoate | 0.0557 | 0.0770 | 0.0631 | 0.0499 | 0.0200 | 0.0393 | 0.0838 | 0.0673 | 0.0125 | 0.1382 | 0.2376 |
| 6. Weight of sublimate, by titration (gram) | | | | 0.0132 | 0.0058 | 0.0103 | 0.0214 | 0.0080 | 0.0015 | 0.0359 | 0.0615 |
| 7. Hence, percentage of benzoic acid | | | | 0.0445 | 0.0195 | 0.0343 | 0.0713 | 0.0530 | 0.0050 | 0.1196 | 0.2037 |
| " " sodium benzoate | | | | 0.0503 | 0.0231 | 0.0403 | 0.0841 | 0.0627 | 0.0063 | 0.1411 | 0.2406 |
| 8. Weight of residue in dish after sublimation (gram) | | 0.0014* | | 0.0023* | 0.0024* | 0.0034* | 0.0016* | 0.0021* | 0.0058* | 0.0093* | 0.0043* |
| 9. Weight of acid in residue, as benzoic, by titration (gram) | 0.0082 | 0.0014 | 0.0061 | 0.0012 | 0.0009 | 0.0024 | 0.0018 | 0.0006 | 0.0015 | 0.0015 | 0.0012 |
| Percentage of benzoic acid | 0.027 | 0.0090 | 0.0203 | 0.0040 | 0.0030 | 0.0080 | 0.0090 | 0.0020 | 0.0050 | 0.0050 | 0.0040 |
| 10. Volatile matter other than sublimate (gram) | | 0.0022 | | 0.0004 | 0.0022 | 0.0010 | 0.0016 | 0.0000 | 0.0000 | 0.0004 | 0.0004 |
| 11. Weight of benzoic acid, by official method (gram) | 0.0263 | 0.0122 | 0.0259 | 0.0161 | 0.0099 | 0.0120 | 0.0255 | 0.0122 | 0.0024 | 0.0373 | 0.0623 |
| 12. Hence, percentage of benzoic acid | 0.0878 | 0.0510 | 0.0862 | 0.0337 | 0.0230 | 0.0400 | 0.0860 | 0.0803 | 0.0080 | 0.1243 | 0.2076 |
| " " sodium benzoate | 0.1027 | 0.0960 | 0.1017 | 0.0630 | 0.0389 | 0.0450 | 0.1020 | 0.0960 | 0.0096 | 0.1466 | 0.2454 |

* Qualitative tests applied to these residues failed to show presence of benzoic acid.

† 0.1401% sodium benzoate added.

‡ 0.2405% sodium benzoate added.

saccharin is evaporated to dryness and the residue is subjected to sublimation instead of to the usual fusion and precipitation, the procedure is shortened and the results are more accurate. Vanillin and coumarin in extracts may be determined by sublimation. In all these examples sublimation may be applied in either the qualitative or quantitative field.

Table 2 gives the results obtained in the determination of benzoic acid both by the official and by the sublimation method, and as will be seen the percentage of benzoic acid obtained by the sublimation method is lower in every case than the percentage obtained by the official method. Neither chloroform nor ether, the solvents commonly used to extract benzoic acid, is absolutely insoluble in water, and it is the opinion of the writers that the water dissolved in these solvents carries with it other organic acids which in the ordinary official method will be titrated and thus give high results. However, if sublimation be resorted to, these impurities will be removed and the results obtained will be more accurate¹.

R. Kemp states that the sensitiveness of the method places it in the same class with the flame color test for sodium compounds and that it is much more accurate than most of the other spectrum colors in the Bunsen flame. If this is true, the smallest specimen, when examined as to form, size, color, temperature of formation, and behavior in polarized light, could be identified with great certainty.

PHOTOMICROGRAPHS.

In the following discussion of photomicrographs the writers wish to give credit to C. S. Corl, formerly of this laboratory, for the sublimation and the photographic work.

All the photomicrographs were taken with the Lucas Photomicrographic camera, which was found to be the most convenient and easy to manipulate; it has the advantage of using an ordinary roll film. The substances are sublimed upon the slides, which are examined under the microscope until one is found that is satisfactory. This slide is placed under the microscope with the camera attachment adjusted and photographed. Several photographs were made of each substance in order to insure the development of at least one clear and properly focused photograph.

The photomicrographs serve as a permanent record of the appearance of the crystals and enable the investigator to make a more complete study of the crystal types of each substance. They also aid in the classification of the crystals into their different systems, which is sometimes a difficult task, and act as a sort of Bertillon system in the identification of unknown compounds.

¹ Randall, *This Journal*, 10, 414 (1927).



COLMARIN
A140

CODINE
A50

COLMESTEROL
A140

CATECHOL
A140



STYRCHONE
A140

SUPHER
A140

UMA
A50

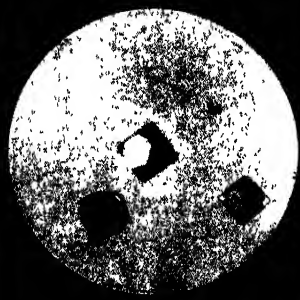
VASELIN
A140



FUMARIC ACID
X50



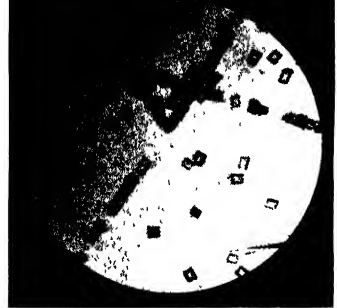
HEXAMETHYLENTETRAMINE
X240



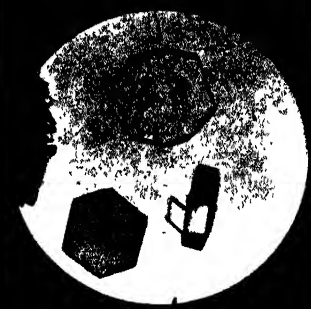
MERCURIC CHLORIDE
X140



MERCURIC CHLORIDE
X140



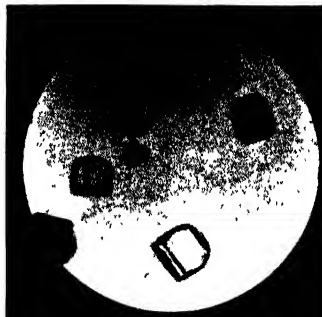
SALTONIN
X50



SALICIN
X140



SALICYLIC ACID
X70



SACCHARIN
X140

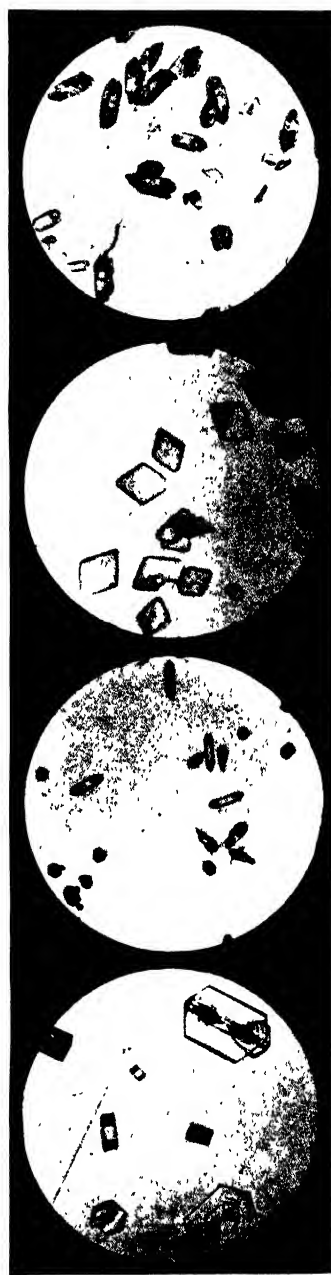


MORPHINE
x140

OXALIC ACID
x50

PYROGALLIC ACID
x140

RESORCIN
x50



CANTHARIDIN
x140

CAFFEINE
x140

B-NAPHTHOL
x140

BENZOIC ACID
x50



ASPIRIN
x140

ANTHRANILIC ACID
x50

ACETANILIDE
x50

ARSENIOUS OXIDE
x50

CONCLUSION.

The results given demonstrate that in all cases the "sublimation point" is lower when determined in vacuo than when determined under atmospheric pressure, and this fact shows clearly that sublimation is dependent upon the vapor pressure. Sublimation is more comparable to evaporation than to boiling, and the phenomenon takes place constantly at all temperatures, indicating that theoretically all substances are capable of being sublimed if a high enough vacuum can be produced. From a practical standpoint, however, the investigator can only be interested in those substances that produce visible sublimates in a reasonable length of time. This work also shows that sublimation can be used to advantage in both quantitative and qualitative analysis.

THE ASSAY OF IPOMEA¹.

By L. E. WARREN (U. S. Food, Drug and Insecticide Administration, Washington, D. C.).

According to the Pharmacopeia of the United States, ipomea is the dried root of *Ipomoea orizabensis* Ledenois (Fam. *Convolvulaceae*). It was introduced as "Mexican Scammony". The British Pharmacopeia of 1914 was the first to make the drug official. A closely related drug, scammony, was official in many editions of the U. S. Pharmacopeia, and it (or its gum-resin) is described in some of the foreign pharmacopeias. At the time of the revision of U. S. P. IX, scammony was no longer available, and ipomea was made official in the U. S. P. X in its place. The therapeutic properties of ipomea are similar in a general way to those of scammony; they are believed to lie entirely in the resin.

Examination of the literature reveals very little information concerning the assay of ipomea. In general, it has been assumed that methods which will serve for the assay of scammony would also be applicable to ipomea.

Flückiger and Hanbury² reported 11.8 per cent of resin from specimens of *I. orizabensis*, but they do not give their method of assay. Deane³ assayed a specimen of ipomea and found 18.5 per cent of resins. The assay method which he used is as follows:

The drug was powdered, exhausted by percolation with alcohol (90 per cent), the greater part of the alcohol was distilled off, and the resulting strong tincture was poured into three times its volume of water. The resin separated as a mass of honey-like consistence. It was well washed with boiling water, until the washings were free from sugar and from color, and then dried on a water bath until the weight was constant.

¹ Presented at the meeting of the American Chemical Society, April, 1930, and published here by courtesy of *Industrial and Engineering Chemistry*.

² *Pharmacographia*, ed. 2, 446 (1879).

³ *False Scammony Root*. *Pharm. J.*, 72, 327 (1904).

The U. S. P. X directs that ipomea shall be assayed by the method that is employed for jalap. This method is also given for the assay of podophyllum. Since the writer had shown that the U. S. P. X methods for the assay of podophyllum¹ and jalap² were not entirely satisfactory, it seemed worth while to ascertain whether the assay of ipomea by the U. S. P. X method would yield analogous results. Also it appeared desirable to try the Jenkins³ method for the assay of podophyllum. This had been found satisfactory for podophyllum by Jenkins³ and by Warren⁴.

The Dale process (for jalap)⁴ and the method (for jalap) recommended by Warren⁵ were included in the study. Further, it was thought best to apply to ipomea the methods for the assay of jalap described in the Dutch Pharmacopeia⁶, the French Pharmacopeia⁷, the German Pharmacopeia⁸, and the Swiss Pharmacopeia⁹. Accordingly each of these methods was considered.

As with jalap, the studies were considered in two phases: (a) processes for extracting the resin, and (b) processes for its purification.

In the Dutch pharmacopeial method (for the assay of jalap), the drug is digested with hot alcohol, and a portion of the filtrate is evaporated. The crude resin is washed once with boiling water, and the residue is dried and weighed. The dried resin is then treated with chloroform to remove certain physiologically inactive constituents, and the insoluble residue is dried and weighed. Since resin of ipomea is completely soluble in chloroform, it is obvious that the method of the Dutch Pharmacopeia for the assay of jalap cannot be applied to ipomea.

In the method of the French Pharmacopeia (for the assay of jalap), the drug is heated with alcohol, a portion of the cooled filtrate is evaporated, and the residue is treated successively with 15 cc. portions of boiling water until the washings become colorless. The washed resin is then dried and weighed.

The German Pharmacopeia directs that the drug (jalap) be macerated with cold alcohol, that a portion of the filtrate be evaporated, and that the residue be washed successively with 20 cc. portions of water at 50°C. The washed resin is then dried and weighed.

The Dale process for washing the resin was found to be unsatisfactory for ipomea, because at 65°C. the resin is too hard for thorough washing. The whole Dale process (i. e., extraction and washing) was, therefore, discarded. The German pharmacopeial method was not satisfactory. This was due in part to the fact that cold alcohol is not a satisfactory

¹ *This Journal*, 10, 272 (1927).

² *Ibid.*, 12, 324 (1929).

³ *J. Ind. Eng. Chem.*, 6, 671 (1914).

⁴ *Pharm. J.*, 119, 316 (1928).

⁵ *This Journal*, 12, 331 (1929).

⁶ *Nederlandsche Pharmacopee*, 5, 375 (1926).

⁷ *Pharmacopée Française*, 365 (1908).

⁸ *Deutsches Arzneibuch*, 6, 725 (1926).

⁹ *Pharmacopoea Helvetica*, 486 (1907).

solvent for resin of ipomea, thus rendering complete extraction of the drug uncertain, and in part to the fact that in water at 50°C. resin of ipomea is not sufficiently soft for thorough washing. The method of the Swiss Pharmacopeia (for jalap) is very similar to the German process. Since the German method was not satisfactory, the Swiss procedure was not tried. It was found that the French pharmacopeial process for jalap was workable for ipomea.

After the preliminary experiments, which eliminated several methods, had been completed, three methods for obtaining and purifying the resin were tried.

The U. S. P. method of extraction and that used in the Jenkins procedure are essentially identical, and they were considered as one. As in the case of jalap, the time for heating was reduced to 30 minutes. Experiments that will not be detailed demonstrated that as a rule the U. S. P. and the Dale extraction processes completely exhaust the drug of resins. Further, when the U. S. P. extraction process for jalap, as shortened in time by Warren, was applied to ipomea, it was found to exhaust the drug of its resin and to be less troublesome to carry out than the Dale extraction process. In the shortened procedure it is essential that the percolation be carried out slowly in order to insure exhaustion of the drug.

As previously mentioned, the U. S. P. X and the Jenkins extraction processes were considered as one. The method used is as follows:

U. S. P. AND JENKINS EXTRACTION PROCESS.

(Modified by reduction in time of heating.)

Place 10 grams of the drug in a No. 60 powder in an Erlenmeyer flask of about 250 cc. capacity and add 50 cc. of alcohol. Fit the flask with a stopper, through which is inserted a glass tube about 2 feet long to act as a reflux condenser, and heat the mixture on a gently simmering steam bath for 30 minutes, shaking occasionally. Transfer the contents of the flask to a small percolator and percolate slowly with warm alcohol until about 95 cc. of tincture has been obtained. Cool the percolate to room temperature and make up the solution to 100 cc. with alcohol.

U. S. P. X METHOD.

Transfer 20 cc. of the tincture of ipomea, prepared as previously described (representing 2 grams of ipomea) to a separator; add 10 cc. of chloroform and 20 cc. of a saturated solution of potassium citrate (20 grams of potassium citrate dissolved in 12 cc. of distilled water). Shake well during 2 minutes, then set aside for not less than 10 hours or overnight. Draw off and discard the lower aqueous liquid, and decant the alcohol-chloroform solution through a small filter wetted with alcohol-chloroform into a tared flask or beaker. Rinse the separator with a mixture of 10 cc. of alcohol and 5 cc. of chloroform, and pass the rinsing through the filter. Mix the chloroformic liquids, evaporate the solution on a water bath, dry the residue at 100°C., and weigh.

JENKINS ASSAY MODIFIED.

Transfer 10 cc. of the tincture of ipomea, prepared as previously described (equivalent to 1 gram of ipomea) to a separator and add 10 cc. of chloroform and 10 cc. of

0.6 per cent hydrochloric acid. Shake the mixture and allow it to separate; draw off the lower layer into another separator, and repeat the extraction of the liquid in the first separator three times, using 15 cc. of a mixture of one volume of alcohol and two volumes of chloroform each time, and adding these extractions to the extractive in the second separator. Shake the combined extractions with 10 cc. of the 0.6 per cent hydrochloric acid and allow the mixture to separate. Draw off the lower layer into a tared flask and repeat the extraction of the acid liquid three times, using 15 cc. of fresh alcohol-chloroform mixture each time. Evaporate the combined alcohol-chloroform extractions, taking care to rotate the container in an inclined position as the last portions of the solvent are dissipated, and dry the residue for 20 minutes at 100°C. Repeat the drying in 20 minute periods until the weight becomes constant or begins to increase.

FRENCH WASHING PROCESS.

Evaporate 25 cc. of the tincture of ipomea, prepared as previously described (representing 2.5 grams of ipomea) to dryness on the water bath in a beaker or flask of suitable size and dry the residue until it is free from alcohol. Add 15 cc. of boiling water and stir the mixture well with a glass rod for 2 minutes to insure thorough washing of the resin. Cool the mixture by placing the container in a jar of cold water and decant the wash water into a 9 cm. filter paper. Repeat the washing of the resin with another 15 cc. portion of boiling water, cooling the mixture after kneading the resin and decanting the washings onto the filter, as described previously. If the second wash water contains much color, it is advisable to wash the resin a third time with boiling water. Dissolve the residue in the container in 15 cc. of warm alcohol and pour the solution onto the filter, collecting the filtrate in a weighed beaker. Use sufficient hot alcohol in small portions to completely transfer the solution of the resin to the filter and insure thorough washing of the filter. Evaporate the combined filtrate and washings, taking care to rotate the container in an inclined position as the last portions of the solvent are dissipated. Dry the residue at 100°C. to constant weight.

Three specimens of ipomea were obtained, two from the Import Office of the Administration and one from an importer. They were authenticated as genuine drug of average quality by J. F. Clevenger, pharmacognocist at the port of New York. The specimens were ground, and the drug was assayed by each of the methods selected for trial by each of several collaborators. The results are given in Table 1.

An examination of the results indicates, as was shown in the case of jalap, that the U. S. P. X and the Jenkins processes tend to give higher results in the assay of ipomea than does the French washing process. However, the difference is not so marked as was the case with jalap. Also some of the results obtained by collaborator C were lower than expected, particularly with sample 3. He suggests that this may be due to incomplete extraction of the drug. All the results obtained by collaborator D are lower than those received from the other collaborators. Believing that it was possible that this may have been due to incomplete extraction, rather than to an intrinsic fault in the method, the writer suggested to this collaborator that he repeat his work. To date only one set of results on the repeat assays has been received. On sample No. 3 by the French washing procedure this worker obtained 15.89, 15.74, 16.11 and 15.86 per cent of resin, or about 2 per cent more than was first reported by him.

TABLE 1.

Analyses of three specimens of ipomea by three processes and by several collaborators.

| SPECIMEN | COLLABORATOR | WASHING PROCESS | | |
|----------|--------------|-----------------------------------|--|---|
| | | Procedure I U. S. P. | Procedure II (Jenkins for Podophyllum) | Procedure III French Pharmacopeia |
| (1) | A | <i>per cent</i> 16 60 16 88 | <i>per cent</i> 16 35 16 45 | <i>per cent</i> 15 75 15 76 |
| | B | 17 54 17 51 | 16 71 16 72 | 15 67 15 61 |
| | C | 15 83 15 45 | 16 69 16 75 | 15 38 15 84 |
| | D | 13 18 12 88 | 12 62 12 48 | 12 30 12 24 |
| (2) | A | 18 73 18 81 | 18 75 18 76 | 17 52 17 54 |
| | B | 19 25 19 28 | 18 93 18 93 | 17.54 17 23 17 29 |
| | C | 18 80 18 35 | 17 81* 17 37* | 18 75 18 95 |
| | D | 15 94 16.40 | 15 94 16 83 16 88 | 15 34 15 83 13 73 |
| (3) | A | 16 07 16 18 | 16 42 16 51 | 15 39 15 39 |
| | B | 15 75 15 74 | 15 88 16 09 16 10 | 14 95 14 92 |
| | C | 13 19* 13 48* | 13 50* 13 20* | 13 72* 13 28* |
| | D | 13 76 14 13 | 14 94 13 64 13 11 | 16 67 13 79 13 90 |

* Note by Collaborator: These percentages are so low I wondered if the extraction had been complete, but lack of time prevented making another longer extraction.

As was done in the case of jalap, experiments were undertaken to determine whether the fractions represented by the difference between some of the values from the first two and the third procedures were physiologically active. Accordingly a quantity of resin of ipomea was prepared from specimen 1 by the U. S. P. X method of assay. The quantity obtained amounted to 2.6188 grams, equivalent to 17.46 per cent of resin. This resin was then washed by the French pharmacopeial

process (for jalap). The resin remaining weighed 2.2231 grams, equivalent to 84.5 per cent of the crude resin taken (14.82 per cent calculated to original drug). The aqueous washings were evaporated to dryness, and the residue was administered to cats. The material had no laxative action. The same experiment was carried out with the same specimen of drug except that the Jenkins process of assay was employed. The resin originally obtained weighed 1.6258 grams, equivalent to 16.26 per cent calculated to the original drug. After being washed the resin weighed 1.4384 grams, equivalent to 88.5 per cent of the crude resin taken (14.38 per cent calculated to the original drug). The aqueous washings were evaporated, and the residue was tested on cats. It had no laxative action. These tests demonstrate that the resin obtained by the U. S. P. assay process and by the Jenkins assay process contains some material that is not found in the resin obtained by the French washing process and which is physiologically inactive. Therefore, it would appear that the results given by the last named process most nearly represent the therapeutic activity of the drug.

As a result of these studies the method given herewith is recommended for the assay of ipomea:

Place 10 grams of the drug in a No. 60 powder in an Erlenmeyer flask of about 250 cc. capacity and add 50 cc. of alcohol. Fit the flask with a stopper, through which is inserted a glass tube about 2 feet long to act as a reflux condenser, and heat the mixture on a gently simmering steam bath for 30 minutes, shaking occasionally. Transfer the contents of the flask to a small percolator and percolate slowly with warm alcohol until about 95 cc. of tincture has been obtained. (To ascertain whether extraction is complete, collect a further 10 cc. of percolate and pour a few drops into cold water; if more than a faint cloudiness appears, continue the percolation with warm alcohol until the test for resin fails. Concentrate the additional percolate by evaporation and add the residue to the flask.) Cool the percolate to room temperature and make up the solution to 100 cc. with alcohol.

Evaporate 25 cc. of the tincture prepared as described (representing 2.5 grams of ipomea) to dryness on the water bath in a beaker or flask of suitable size and dry the residue until it is free from alcohol. Add 15 cc. of boiling water and stir the mixture well with a glass rod for 2 minutes to insure thorough washing of the resin. Cool the mixture by placing the container in a jar of cold water and decant the wash water into a 9 cm. filter paper. Repeat the washing of the resin with another 15 cc. portion of boiling water, cooling the mixture after kneading the resin and decanting the washings onto the filter, as described previously. If the second wash water is more than slightly colored, it is advisable to wash the resin a third time with boiling water. Dissolve the residue in the container in 15 cc. of warm alcohol and pour the solution onto the filter, collecting the filtrate in a weighed beaker. Use sufficient hot alcohol in small portions to completely transfer the solution of the resin to the filter and insure thorough washing of the filter. Evaporate the combined filtrate and washings, taking care to rotate the container in an inclined position as the last portions of the solvent are dissipated. Dry the residue at 100°C. to constant weight.

ACKNOWLEDGMENTS.

The writer wishes to acknowledge his appreciation to the several teachers and students connected with schools of pharmacy who have aided in this study by collaborative assays; also to J. L. Hopkins and Company and to the New York Station of the Food, Drug and Insecticide Administration for furnishing and authenticating the crude drugs. Thanks are also due to the staff of the Pharmacological Laboratory of the Food, Drug and Insecticide Administration for ascertaining the physiological activity of the several fractions of resin of ipomea on cats and to William R. Carter for carrying out a number of the routine determinations and for checking many of the other analyses.

THE BIOASSAY OF CAPSICUM, U. S. P. X¹.

By JAMES C. MUNCH (Sharp and Dohme, Baltimore, Md.).

The U. S. Pharmacopeia X defines capsicum as "the dried ripe fruit of *Capsicum frutescens* Linné (Fam. *Solanaceae*), grown in Africa". It differs from condimental red pepper in several particulars, the chief distinction being its pronounced pungency. The Pharmacopeia specifies that a distinct sensation of pungency is produced in the throat of at least two out of three individuals on swallowing 5 cc. of a solution representing 14.3 mg. of crude drug, or 2.86 mg. of oleoresin, extracted with 95 per cent alcohol and diluted with 10 per cent sucrose. Since this method does not recognize the great variation in perceptivity of different individuals, it is essentially a qualitative rather than a quantitative procedure.

Through the kindness of E. K. Nelson, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C., a sample of capsaicin, which he had extracted from capsicum, was obtained². A solution was made in dilute alcohol, and diluted with 10 per cent sucrose; 5 cc. of each dilution was swallowed by 94 different individuals, and the degree of pungency was noted. The results are reported in Table 1. Zero represents no apparent pungency; 1 plus, a just detectable positive reaction; and the greater responses are represented by 2, 3 and by 4 plus, which is the maximum reaction. The variability of perceptivity of individuals is readily recognized in these results. At a concentration of 1 : 10,000,000 or 0.1 mg. per liter, half of the tests were positive and half were negative. This concentration had been reported³ as the threshold of detectable pungency. Significant correlation was

¹ Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1929.

² *J. Ind. Eng. Chem.*, 2, 419 (1910); *J. Am. Chem. Soc.*, 41, 1115 (1919); 42, 597 (1920); 45, 2179 (1923).

³ Gathercoal and Terry, *J. Am. Pharm. Assoc.*, 10, 423 (1921); Wirth and Gathercoal, *Ibid.*, 13, 217 (1924).

found between concentration of capsaicin and degree of response ($r = 0.65 \pm 0.04$).

It is obviously necessary, in making this method a quantitative procedure, to adopt some common standard for evaluating individual sensitivity to pungent substances. Capsaicin would be very satisfactory, but it is not available in sufficient quantities. After a number of other substances had been tested, it was found that piperine was well suited for use as a standard. Solutions containing 15 mg. per liter failed to give any response, whereas solutions containing 16 mg. per liter showed definite pungency in the throats of ten individuals. Three lots of piperine had the same pungency.

The work of Gathercoal, Terry and Wirth amplified studies by Scoville¹. A detailed study of the effect upon the ultimate pungency of modifying different steps in procedure led to the development by the writer² of the following bioassay method:

Shake 1 gram of coarsely powdered capsicum with 50 cc. of alcohol in a stoppered flask for 3 hours. Dilute 0.1 cc. of the clear, supernatant liquid with 100 cc. of 10 per cent sucrose solution. Swallow 5 cc. of this dilution during 5 seconds. The same degree of pungency should be experienced in the throat as that produced by 5 cc. of 10 per cent sucrose solution containing 16 mg. of piperine per liter. In case 16 mg. of piperine per liter does not produce satisfactory pungency, determine the threshold concentration and make corresponding alterations in the standard for capsicum (0.1 cc. per 100 cc. or 20 mg. per liter).

Using this method, the writer determined the pungency of a number of samples of capsicum and of its oleoresin. Some of the results are reported in Table 2. No samples of condimental red pepper or tabasco met the U. S. P. requirement for pungency. A number of samples obtained from crude drug brokers in response to requests for "Ground Capsicum, U. S. P.", were all substandard in pungency, indicating inferiority or adulteration. Only one of four commercial oleoresins complied with standard requirements. Oleoresins prepared from Ameri-

TABLE 1.
Pungency of capsaicin to individuals.

| DEGREE OF PUNGENCY | CONCENTRATION OF CAPSAICIN—MG. PER LITER | | | | | | | | | |
|-----------------------|--|------|-------|------|------|-------|------|-----|------|-----|
| | 0.075 | 0.10 | 0.125 | 0.13 | 0.16 | 0.175 | 0.25 | 1.0 | 1.25 | 2.5 |
| 0 | 21 | 47 | 3 | 0 | 0 | 0 | 1 | .. | .. | .. |
| 1 plus | .. | 47 | 12 | 2 | 2 | 1 | 5 | 3 | 1 | .. |
| 2 plus | .. | .. | 1 | .. | .. | 1 | 8 | 5 | .. | 3 |
| 3 plus | .. | .. | .. | .. | .. | .. | 2 | 5 | .. | 1 |
| 4 plus | .. | .. | .. | .. | .. | .. | .. | .. | .. | 6 |
| Total | 21 | 94 | 16 | 2 | 2 | 2 | 16 | 13 | 1 | 10 |

¹ *J. Am. Pharm. Assoc.*, 1, 453 (1912).

² *Ibid.*, 18, 1236 (1929).

can grown "Louisiana Sports" and from Japan Chillies were very weak in pungency.

It is suggested that attention be directed to the bioassay of capsicum to distinguish between capsicum, U. S. P. (medicinal), and the other varieties which are used as condiments, but are essentially non-pungent. Many commercial samples of capsicum and of its oleoresin are adulterated with these non-pungent varieties. This bioassay method readily serves to detect such adulteration.

TABLE 2.
Pungency of capsicum and its oleoresin.

| PA NO. | PRODUCT | MINIMUM EFFECTIVE CONCENTRATION |
|--------------|--------------------------------------|---------------------------------------|
| | CAPSICUM | <i>mg./liter</i> |
| 480 | Zanzibar | 20 |
| 481 | Sierra Leone | 20 |
| 695 | Sierra Leone | 30 |
| 496 | Annuum | 30 |
| 499 | Annuum | 30 |
| 692 | Japan chillies | 30 |
| 688 | Louisiana sports | 200 |
| 689 | Louisiana longs | 400 |
| 687 | Tobasco | 100 |
| 682 | Short Bombay | 100 |
| 683 | Long Bombay | 140 |
| 649 | Commercial sample No. 1 | 30 |
| 650 | Commercial sample No. 2 | 20 |
| 651 | Commercial sample No. 3 | 60 |
| | OLEORESIN | |
| 679 | Stock oleoresin | 3 0 |
| 673-678, 686 | 7 stock oleoresins | 3 5 |
| 672 | Commercial sample No. 3 | 3 5 |
| 673 | Commercial sample No. 4 | 5 0 |
| 670 | Commercial sample No. 1 | 7 0 |
| 671 | Commercial sample No. 2 | 10 0 |
| 723 | Louisiana sports oleoresin | 12 0 |
| 690 | Japan chillies | 20 0 |

DETERMINATION OF CREAM OF TARTAR AND TARTARIC ACID IN TARTRATE BAKING POWDERS.

By B. G. HARTMANN (Food Control Laboratory¹, Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.).

The official and tentative methods of analysis of the Association of Official Agricultural Chemists describe the determination of total tartaric acid in baking powders², but no quantitative procedures for the

¹ W. B. White, Chief, Food Control.

² *Methods of Analysis*, A. O. A. C.

determination of cream of tartar and tartaric acid in these products are available.

After extensive experimentation the writer devised the following methods for the determination of these constituents. These methods are rapid and also accurate.

TOTAL TARTARIC ACID, CREAM OF TARTAR AND TARTARIC ACID.

Transfer 2.5 grams of the baking powder to a 250 cc. volumetric flask, add 100 cc. of distilled water of 50°C., and allow the mixture to stand 30 minutes, shaking occasionally. Make to mark with distilled water, shake, and run through a folded filter into a dry flask. Measure two portions of 100 cc. each of the filtrate into 250 cc. beakers and evaporate to 20 cc. To one portion add 3.5 cc. of normal *potassium* hydroxide, 2 cc. of glacial acetic acid and 80 cc. of 95 per cent alcohol. Treat the other portion in a similar manner, but use normal sodium hydroxide instead of normal potassium hydroxide. Place the beakers in a refrigerator for about 1 hour, stir vigorously for 2 minutes, and allow to remain in the refrigerator overnight. Collect the precipitate in a Gooch crucible on a thin, tightly tamped pad of asbestos. Wash the beaker thoroughly with ice-cold 80 per cent alcohol and finally wash the precipitate several times with the cold alcohol. Transfer the contents of the Gooch to the original beaker with hot water, heat to boiling, and titrate with 0.1 *N* alkali, using phenolphthalein as indicator. Designate the titration of the portion treated with potassium hydroxide as "A" and that treated with sodium hydroxide as "B".

Calculations:

Per cent Total Tartaric Acid—

$$\frac{0.015(A) \times 2.5 \times 100}{2.5} \text{ or } 1.5 A;$$

Per cent Cream of Tartar—

$$\frac{0.0188(B) \times 2.5 \times 100}{2.5} \text{ or } 1.88 B; \text{ and}$$

Per cent Tartaric Acid—

$$\frac{0.015 (A - B) \times 2.5 \times 100}{2.5} \text{ or } 1.5 (A - B).$$

The procedure was tried on baking powders prepared in the laboratory.

The results obtained on commercial preparations are given in Table 2. In a baking powder containing a slight excess of sodium bicarbonate, the neutralizing value is an index of the available carbon dioxide of the powder. The last two columns record the neutralizing value (apparent

TABLE 1.

Results on laboratory preparations.

| SAMPLE NO. | CONTAINED | | | DETERMINED | | | A | B |
|------------|-------------------------|-------------------------|----------------------|-------------------------|-------------------------|------------------------|-------|-------|
| | Total Tartaric Acid | Cream of Tartar | Tartaric Acid | Total Tartaric Acid | Cream of Tartar | Tartaric Acid | | |
| 1 | <i>per cent</i> 47 9 | <i>per cent</i> 60 0 | <i>per cent</i> 0 | <i>per cent</i> 48 5 | <i>per cent</i> 60 2 | <i>per cent</i> 0 5 | 32 30 | 32 00 |
| 2 | 23 6 | 0 | 23 6 | 23 3 | 0 6 | 22 8 | 15 50 | 0 30 |
| 3 | 26 9 | 5 9 | 21 2 | 25 3 | 5 3 | 21 1 | 16 85 | 2 80 |
| 4 | 41 6 | 44 7 | 5 9 | 40 4 | 44 4 | 5 0 | 26 95 | 23 60 |
| 5 | 35 3 | 29 5 | 11 8 | 34 4 | 29 4 | 11 0 | 22 95 | 15 65 |

available carbon dioxide) and the available carbon dioxide determined by the gasometric method¹. The neutralizing value was calculated by the following formula: Cream of Tartar \times 0.234 + Tartaric Acid \times 0.527.

TABLE 2.

Results on commercial preparations.

| SAMPLE NO | A | B | TOTAL TARTARIC ACID | CREAM OF TARTAR | TARTARIC ACID | NEUTRALIZ-ING VALUE | AVAILABLE CO ₂ GASOMETRIC* |
|-----------|----------------|----------------|-------------------------|-------------------------|------------------------|---------------------|---------------------------------------|
| 1 | 25.95 | 23 4 | <i>per cent</i> 38 9 | <i>per cent</i> 41 0 | <i>per cent</i> 3 8 | 12 5 | 13 1 |
| 2 | 22.80 | 16 1 | 34 2 | 30 3 | 10 1 | 13 0 | 13 2 |
| 3 | 18 25 | 8 5 | 27 4 | 16 0 | 14 6 | 12 3 | 13 2 |
| 4 | 29.00 29 10 | 27.75 27 85 | 43 5 43 7 | 52 2 52 4 | 1 9 1 9 | 13 3 13 4 | 12 8 |
| 5 | 21.0 | 11.20 | 31 5 | 21 1 | 14 7 | 13 6 | 13 2 |
| 6 | 25 50 25 50 | 24 30 24 20 | 38 3 38.3 | 45 7 45 5 | 1 8 2 0 | 11 8 11 8 | 12 5 |

* Determined by V. E. Munsey, Food Control Laboratory.

DIRECT DETERMINATION OF TARTARIC ACID.

Transfer 1.25 grams of baking powder to a dry 200 cc. measuring flask, add 50 cc. of chloroform, and allow to stand 5 minutes, shaking occasionally. Then add 100 cc. of alcohol saturated with cream of tartar and allow to stand 30 minutes, shaking occasionally. (The cream of tartar should be absolutely free of tartaric acid. Wash the c. p. salt

¹ *Methods of Analysis*, A. O. A. C.

repeatedly with distilled water, then with alcohol, and finally with ether. Dry at the temperature of boiling water. The saturated alcohol is prepared by adding about 20 grams of washed and dried cream of tartar to 1 liter of absolute alcohol, shaking vigorously for several minutes, and allowing to stand 1 hour with occasional shaking. Shake immediately before using.) Make to mark with the alcohol, shake, and filter. Titrate 100 cc. of the filtrate with 0.1 *N* alkali, using phenolphthalein as indicator. Designate the alkali used as "A" and calculate the percentage of tartaric acid in the baking powder by the following formula:

$$\text{Per cent Tartaric Acid} = \frac{2 A \times 0.0075 \times 100}{1.25} \text{ or } 1.2 A.$$

The method was tried on baking powders prepared in the laboratory. The results are given in Table 3.

TABLE 3.
Results on laboratory preparations.

| SAMPLE NO. | CONTAINED | | DETERMINED | |
|------------|-----------------|-----------------|-----------------|-----------------|
| | Cream of Tartar | Tartaric Acid | Cream of Tartar | Tartaric Acid |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1 | 59 5 | 0 | | 0 2 |
| 2 | 56 6 | 1 0 | | 0 8 |
| 3 | 45 1 | 5 8 | | 5 4 |
| 4 | 29 8 | 11 8 | | 11 2 |
| 5 | 6 0 | 21 2 | | 20 6 |
| 6 | 0 | 23 5 | | 23 2 |

The procedure was tried on the six commercial powders. For comparative purposes the results obtained by the precipitation method are recorded in Table 4.

TABLE 4.
Results on commercial preparations.

| SAMPLE NO. | TARTARIC ACID | |
|------------|-----------------|-----------------|
| | Direct | Indirect |
| | <i>per cent</i> | <i>per cent</i> |
| 1 | 3.7 | 3.8 |
| 2 | 10 4 | 10.1 |
| 3 | 14.8 | 14 6 |
| 4 | 0 4 0 4 | 1 9 1.9 |
| 5 | 15 0 | 14 7 |
| 6 | 1.0 0.9 | 1 8 2.0 |

The results obtained by the two methods are in good agreement. An exception is noted in the case of sample No. 4. This product had a grayish flaky appearance and a strong abnormal odor resembling lye. When 10 grams of the powder was treated with alcohol-chloroform and the resulting solution was evaporated to dryness, no tartaric acid crystals were discernible under the microscope. Treated in a like manner, sample No. 6 yielded positive evidence of the presence of tartaric acid, as did all the other samples.

SUMMARY.

Two procedures are described: (1) The determination of the tartrate constituents of tartrate baking powders, and (2) the direct determination of tartaric acid in these products. The data obtained in the first procedure also provide for the determination of the apparent available carbon dioxide.

IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS.

I. OPTICAL PROPERTIES OF THE SEMICARBAZONES OF CERTAIN ALDEHYDES AND KETONES.

By JOHN B. WILSON and GEORGE L. KEENAN¹.

In the chemical examination of flavors, the analyst is frequently required to establish the identity of aldehydes and ketones that have been added in minute quantities for the purpose of increasing the intensity of the aroma to a point where the manufactured product can be diluted to a much greater extent than is possible with the unadulterated natural product.

The aldehydes and ketones in essential oils and in other natural products containing substantial proportions of these substances are usually separated by treating the product with some reagent that will form a relatively stable, insoluble compound. This insoluble compound is then purified, if necessary, and identified by its melting point. The semicarbazones are very useful for this purpose because they can usually be prepared with ease and recrystallized in a pure condition. Veibel², however, has shown that the melting points of such substances are subject to wide variations, depending upon the rate of heating. He devised a method for the identification of aldehydes and ketones in which the semicarbazones are precipitated and purified, and one atom of nitrogen per molecule is determined as ammonia. This method

¹ Joint contribution from the Water and Beverage Unit of Food Control, and Microanalytical Laboratory, Food and Drug Administration, U. S. Department of Agriculture.

² *Bull. soc. chim.*, 4th ser., 41, 1410 (1927).

requires 0.3 gram or more of the semicarbazone to obtain duplicate determinations of nitrogen, or even a larger quantity in the case of substances having a high molecular weight.

In view of the very small proportions of aldehydes, ketones and other synthetic substances used in the manufacture of flavors and beverage concentrates, Veibel's method is not always applicable; it is also open to the objection that frequently more than one compound may have the same content of nitrogen. For example, each of the semicarbazones of citral, thujone, and vanillin contains 6.70 per cent of nitrogen, as determined by Veibel's method, while each of the semicarbazones of carvone and heliotropine determined by the same method contains 6.76 per cent of nitrogen. Moreover, the nitrogen content in these cases is almost identical, a disconcerting fact, since vanillin and heliotropine may be found together in the same flavor. While in the case of essential oils, the composition of which has been widely studied, there might be little if any difficulty in differentiating compounds yielding semicarbazones with substantially the same nitrogen content, greater difficulty would be encountered in the analysis of unknown mixtures such as the flavors.

Because the writers were aware of the limitations of the former methods for the identification of the semicarbazones, they used the optical immersion method commonly employed by petrographers in the study of minerals and more recently applied to the examination of inorganic and organic crystalline substances. This method has been described many times elsewhere, but a brief summary will be given later.

PREPARATION OF THE SEMICARBAZONES.

Reagent.—The reagent used was made by dissolving 11.2 grams of semicarbazide hydrochloride and 12.5 grams of anhydrous sodium acetate in about 80 cc. of hot water. The mixture was filtered into a 100 cc. volumetric flask, and the beaker and filter paper were washed with small portions of hot water until the liquid in the flask reached nearly to the mark. The solution was then cooled to room temperature and made up to the mark with water. It was about normal in precipitating strength.

Semicarbazones.—From 0.50 to 1.00 gram of the aldehyde or ketone was weighed and dissolved in 5–10 cc. of alcohol, 10 cc. of the reagent was added, and the mixture was allowed to stand until crystals began to form. When the precipitation appeared to be well advanced, about 25–50 cc. of water was added, and the mixture was allowed to stand overnight; the crystals were then filtered off and dried at 100°C. The chemical analysis and other data were obtained on the semicarbazones prepared in this manner.

Later, in order to ascertain whether the crystal form remained the same when only small quantities of the aldehydes and ketones were used, each of the compounds was prepared at least twice in the following manner: From 0.05 to 0.2 gram of the aldehyde or ketone was dissolved in 2 cc. of alcohol, 2 cc. of the reagent was added, and the mixture was allowed to stand 30 minutes, or longer if necessary, until crystals began to form. Water up to 2 cc. was then added dropwise. If the solution became cloudy due to the aldehyde or ketone separating out, it was left to stand longer; if not, 25 cc. of water was added, and the solution was left for several hours. It was then filtered, and the semicarbazone was washed with water and dried at 100°C. on the filter paper. Since the optical properties were the same, this method is recommended when only a small quantity of the substance is available.

MELTING POINT.

The melting point was determined in a capillary tube by heating the substance rapidly to within 10° of the expected melting point, then more slowly until the melting temperature had been attained.

NITROGEN.

(Veibel's method for determining one atom of nitrogen in semicarbazones.)

The following reagents were used: (1) *Potassium iodate solution*, prepared by dissolving 5 grams of potassium iodate in water and diluting to 100 cc.; (2) *sodium hydroxide solution*, prepared by dissolving 400 grams of sodium hydroxide in water and diluting to 1 liter.

From 0.10 to 0.20 gram of the semicarbazone was placed in a Kjeldahl flask, 100 cc. of water and 10 cc. of sulfuric acid were added, and the preparation was boiled under a reflux condenser for 30 minutes, or until all the solids were in solution. (In the case of difficultly decomposable compounds, 30 cc. of water was used.) Then 10 cc. of potassium iodate solution was added, and the mixture was boiled until the iodine color had disappeared. The solution was then cooled, 100 cc. of water and 50 cc. of sodium hydroxide solution were added, and about 150 cc. was distilled off into 25 cc. of standard acid. The distillate was titrated back, methyl red indicator being used, and the percentage of nitrogen was calculated.

MOLECULAR WEIGHT.

After one atom of nitrogen had been determined by the above method, the molecular weight of the aldehyde or ketone was calculated by the following formula:

$$\text{Mol. wt.} = \frac{1400.8}{\text{Percentage of nitrogen found}} - 57.05.$$

OPTICAL PROPERTIES.

The method adopted requires the use of a petrographic or chemical microscope that is equipped with an analyzing and polarizing nicol in addition to the other accessories with which such an instrument is provided. The worker is thus enabled to study crystalline material, such as the semicarbazones about to be described, in ordinary light, in parallel polarized light (crossed nicols), and in convergent polarized light (crossed nicols).

In studying any of the crystalline semicarbazones by this method, a small quantity of the material is finely powdered and immersed in a drop of oily liquid on a microscopical slide. The indices of refraction of the liquids used should be determined previously on a refractometer. The liquids most suitable for immersion purposes consist of mixtures of the following: mineral oil ($n = 1.49$), monochloronaphthalene ($n = 1.64$), monobromnaphthalene ($n = 1.66$), and methylene iodide ($n = 1.74$), so prepared that each will differ in refractive index from the next by 0.005. Additional liquids with smaller increments can be made as the occasion requires.

As already indicated, the crystalline fragments are successively immersed in the liquids for the purpose of measuring their refractive indices, these being the most useful of the optical properties for this purpose. In this method reliance is placed on the fact that the greater the difference in the refractive indices of liquid and crystalline fragment, the more distinctly the crystal will stand out in the menstruum. By repeated trials in different liquids, there will be found ultimately one in which the margin of the crystal will entirely disappear. If crystals or fragments are placed in different positions with reference to the plane of vibration of the nicol and successively immersed in other liquids, one, two, or three indices of refraction, as the case may be, can be determined. Usually, only the minimum and maximum values are necessary for identifying the substance.

Substances crystallizing in the cubic system show but one index of refraction, transmit no light when examined with crossed nicols and are said to be singly refractive. Many other substances, like the semicarbazones to be described, are doubly refractive and do transmit light when the nicols are crossed and the stage rotated. As many as three indices of refraction, designated as n_α , n_β , and n_γ , may be determined on such substances. The semicarbazones examined belong to this group of doubly refractive materials.

Other data may be obtained in convergent polarized light (crossed nicols), but these are not so important for determinative work as the refractive indices.



FIG. 1. ACETOPHENONE SEMICARBAZONE
(X100)



FIG. 2. ANISIC ALDEHYDE SEMICARBAZONE
(X250)



FIG. 3. BENZALDEHYDE SEMICARBAZONE
(X180)

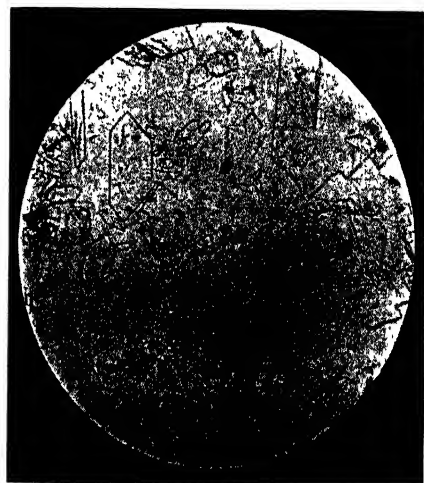


FIG. 4. BENZALIDINE ACETONE SEMICARBAZONE
(X100)

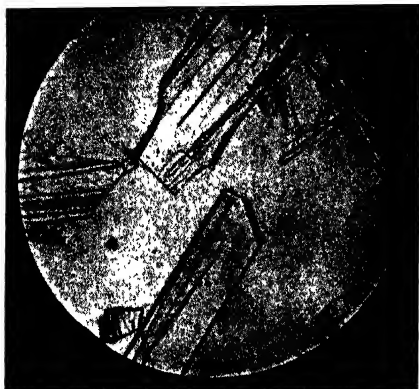


FIG. 5. CARYONE SEMICARBAZONE
(X70)



FIG. 6. ETHYL-PROTO CATECHUIC ALDEHYDE
SEMICARBAZONE
(X200)



FIG. 7. *p*-Methyl-acetophenone Semicarbazone
(X180)



FIG. 8. β -THUJONE SEMICARBAZONE
(X120)



FIG. 9. VANILLIN SEMICARBAZONE
(X110)

DESCRIPTION OF SEMICARBAZONES.

Acetophenone Semicarbazone.—Crystals began to separate within a few minutes, and within 45 minutes the mixture had become nearly solid. Analysis: Nitrogen 7.96 per cent, theory 7.91 per cent; molecular weight 118.93, theory 120.06; m. p. 197°C. (195°–198°C.¹).

Optical Properties: This material occurs as thin, platy, micaceous crystals, some of which are irregularly six-sided, others rod-shaped. The indices of refraction are: $n_\alpha = 1.480$ (common on irregular fragments and crosswise on rods); n_β = indeterminate; $n_\gamma = 1.660$ (common on irregular fragments and lengthwise on rods); both ± 0.003 . All the plates extinguish sharply with crossed nicols, indicating that β is more or less perpendicular to the broad face and therefore interference figures could not be expected. The extinction is straight and the sign of elongation + on the rods (Fig. 1.)².

Anisic Aldehyde Semicarbazone.—Crystals began to appear within 3–5 minutes, and within 10 minutes the crystallization appeared to be complete. Analysis: Nitrogen 7.29 per cent, theory 7.25 per cent; molecular weight 135.10, theory 136.06; m. p. 210°C. (203°–210°C.).

Optical Properties: Crystallizes in thin, colorless plates, which do not extinguish with crossed nicols but remain practically bright when the stage is rotated. Plates showing a biaxial interference figure with the optic axis up are common when the substance is examined in convergent polarized light (crossed nicols). The indices of refraction are: $n_\alpha = 1.653$ (not common, and on plates extinguishing sharply); $n_\beta = 1.692$ (common on plates not extinguishing sharply); $n_\gamma = > 1.736$; both ± 0.003 . (Fig. 2.)

Benzaldehyde Semicarbazone.—Crystals formed at once becoming almost solid in 2 minutes. Analysis: Nitrogen 8.53 per cent, theory 8.59 per cent; molecular weight 107.17, theory 106.05; m. p. 217°C. (214°–235°C.).

Optical Properties: This substance crystallizes in elongated plates with jagged edges and frequently with notched ends. The plates do not extinguish sharply with crossed nicols and show one optic axis up in the biaxial interference figure, permitting of the ready determination of the β -value. The extinction is straight and the sign of elongation The indices of refraction are: $n_\alpha = 1.560$ (not common and often difficult to locate); $n_\beta = 1.685$ (common); n_γ = very high and immeasurable by this method; both ± 0.003 . (Fig. 3.)

Benzylidene Acetone Semicarbazone.—Crystals began to separate within 5 minutes, while the whole became a solid mass of crystals within 30 minutes. Analysis: Nitrogen 6.84 per cent, theory 6.89 per cent; molecular weight 147.74, theory 146.08; m. p. 186°C. (185°–187°C.).

¹ The figures in parentheses were obtained from Beilstein's *Organische Chemie*, 4th ed.

² The crystal forms illustrated do not always remain intact when the material is studied optically. It is usually necessary to crush the substance for optical study, thereby obtaining irregular fragments.

Optical Properties: To the naked eye this semicarbazone is of a lemon-yellow color. Under the microscope it consists of platy material, many of the fragments being six-sided and some of them elongated, the latter showing straight extinction. Only partial biaxial interference figures are shown in convergent polarized light (crossed nicols). The indices of refraction are: $n_{\alpha} = 1.450$ (common on plates and crosswise on elongated forms); $n_{\beta} = 1.618$ (common lengthwise on elongated forms); $n_{\gamma} = > 1.736$; both ± 0.003 . (Fig. 4.)

Carvone Semicarbazone.—There was no sign of a precipitate after 3 hours, but a fair amount of the crystals had been deposited after standing overnight. After adding 2 cc. more of the reagent and 10 cc. of water the crystals began to form rapidly and the crystallization appeared to be complete after about 3 hours. Analysis: Nitrogen 6.75 per cent, theory 6.76 per cent; molecular weight 150.47, theory 150.11; m. p. 143°C . (141° – 142°C .).

Optical Properties: This semicarbazone consists of rod-shaped crystals and irregular fragments. In convergent polarized light (crossed nicols), partial biaxial interference figures showing one optic axis up are common. The indices of refraction are: $n_{\alpha} = 1.490$ (common); $n_{\beta} = 1.645$ (common); $n_{\gamma} = 1.710$ (common); all ± 0.003 . (Fig. 5.)

Citral Semicarbazone.—Precipitation began within 1 minute and appeared to be complete within 10 minutes. Analysis: Nitrogen 6.48 per cent, theory 6.70 per cent; molecular weight 159.12, theory 152.12; m. p. 132°C . (135°C .).

Optical Properties: Crystallizes in thin, platy crystals without a significant habit. The plates invariably extinguish sharply with crossed nicols (parallel polarized light). The refractive indices are: $n_{\alpha} = 1.560$ (common); $n_{\beta} = \text{indeterminate}$; $n_{\gamma} = 1.660$; both ± 0.003 .

Ethyl-protocatechuic Aldehyde Semicarbazone.—A quantity of ethyl-protocatechuic aldehyde (0.075 gram) was dissolved in 2 cc. of alcohol, and 2 cc. of semicarbazide reagent was added. Crystals began to appear within 10 minutes. After 20 cc. of water had been added, the crystals came down faster. Analysis: Nitrogen 6.28 per cent, 6.27 per cent, 6.30 per cent, theory 6.28 per cent; molecular weight 165.65, theory 166.08; m. p. 175°C . (Not found in the literature.)

Optical Properties: This material consists of irregular fragments, some of which have a fibrous appearance. Partial biaxial interference figures are shown in convergent polarized light (crossed nicols). The indices of refraction are: $n_{\alpha} = 1.445$; $n_{\beta} = \text{indeterminate}$; $n_{\gamma} = > 1.736$. An intermediate refractive index, $n_i = 1.690$, occurs very frequently and is significant for determinative purposes; both ± 0.003 . (Fig. 6.)

Heliotropine Semicarbazone.—Crystals began to appear within 1 minute and within 5 minutes the mixture had become a solid mass of crystals.

Analysis: Nitrogen 6.76 per cent, theory 6.76 per cent; molecular weight 150.17, theory 150.05; m. p. 234°C.

Note.—There appears to be a disagreement in the literature regarding the melting point of this substance. Parry gives the figure 146°C. in his "Chemistry of Essential Oils and Artificial Perfumes". Gildemeister and Hoffman give 224°C. as the melting point of the semicarbazone of heliotropine (piperonal) in "Die Atherische Ole, III Auflage, 1928", while in the textbook "Organic Analysis, Qualitative and Quantitative", by E. de Barry Barnett and F. C. L. Thorne, the figure 228°C. is given.

Optical Properties: This semicarbazone consists of thin, rectangular plates, and occasionally of rods. The extinction is straight on the rods and the sign of elongation —. All the plates extinguish sharply, indicating that β is more or less perpendicular to the broad face, so that interference figures would not be expected. The refractive indices are: $n_\alpha = 1.580$ (common); n_β = indeterminate; $n_\gamma = 1.725$; both ± 0.003 .

***l*-Menthone Semicarbazone.**—Since no precipitate appeared within 30 minutes after 3 drops of *l*-menthone was dissolved in 2 cc. of alcohol and treated with 2 cc. of semicarbazide reagent, 1 cc. of water was added and then a precipitate began to form at once. **Analysis:** Nitrogen 6.63 per cent, theory 6.63 per cent; molecular weight 154.23, theory 154.14; m. p. 184°C. (184°C.).

Optical Properties: Crystallizes in the form of irregular fragments and rods. The fragments usually extinguish sharply with crossed nicols (parallel polarized light). The indices of refraction are: $n_\alpha = 1.528$ (common on fragments and crosswise on rods); n_β = indeterminate; $n_\gamma = 1.590$ (common on fragments and lengthwise on rods); both ± 0.003 .

Para-methyl-acetophenone Semicarbazone.—The crystals formed very slowly but the crystallization appeared to be complete after 2 hours. **Analysis:** Nitrogen 7.38 per cent, theory 7.32 per cent; molecular weight 132.76, theory 134.08; m. p. 210°C. (205°C.).

Optical Properties: Crystallizes in rods, usually six-sided in outline, and sometimes occurring in lath-like aggregates. The extinction is straight and the sign of elongation . . . The material is biaxial, but no interference figures are visible on most of the plates. The indices of refraction are: $n_\alpha = 1.445$ (always shown crosswise and common); n_β = indeterminate; $n_\gamma = 1.645$ (always shown lengthwise and common); both ± 0.003 . (Fig. 7.)

Methyl-undecyl Ketone Semicarbazone.—Crystals formed at once and appeared to be complete within 30 minutes. **Analysis:** Nitrogen 5.51 per cent, theory 5.49 per cent; molecular weight 197.18, theory 198.20; m. p. 123°C. (123°C.).

Optical Properties: This semicarbazone consists of narrow, thin, lath-like plates with jagged edges. It is characteristic of the plates to remain on edge, giving the appearance of needles. The indices of refrac-

tion are: $n_\alpha = 1.480$ (common and shown crosswise on plates on edge); $n_\beta = 1.560$ (very common, and lengthwise on plates on edge); $n_\gamma = 1.580$ (not common); all ± 0.003 . (The value $n = 1.560$ apparently is the β -value, although lack of a satisfactory interference figure could not positively confirm this.)

Beta-thujone Semicarbazone.—No precipitate formed within 30 minutes, and 1 cc. of water was added. After the solution had stood overnight a precipitate was formed. Analysis: Nitrogen 6.71 per cent, theory 6.70 per cent; molecular weight 151.71, theory 153.13; m. p. 170°C . (174°C .).

Optical Properties: The material usually breaks up into irregular fragments without any significant habit when crushed for optical study. Elongated and dagger-shaped forms occur occasionally, the former showing straight extinction and positive elongation. The indices of refraction are: $n_\alpha = 1.520$ (common); $n_\beta =$ indeterminate; $n_\gamma = 1.590$ (common); both ± 0.003 . (Fig. 8.)

Vanillin Semicarbazone.—Crystals began to form within 3–4 minutes, and the precipitation appeared to be complete within 30 minutes. Analysis: Nitrogen 6.70 per cent, theory 6.70 per cent; molecular weight 152.03, theory 152.06; m. p. 230°C . (229°C .).

Optical Properties: Crystallizes in rods and plates. The extinction is straight and the sign of elongation is —. The indices of refraction are: $n_\alpha = 1.692$ (common lengthwise on rods); n_β and n_γ are both higher than 1.736 and could not be measured by this method. (Fig. 9.)

Summary of determinative data.

| SEMICARBAZONE | N. | MOL. WT. | M. P. | α | β | γ | COMMON N |
|------------------------------------|------|----------|-------------|----------|---------|-----------|------------|
| Acetophenone..... | 7.91 | 120.06 | 197° | 1.480 | indet. | 1.660 | both |
| Anisic aldehyde..... | 7.25 | 136.06 | 210° | 1.653 | 1.692 | 1.736 | β |
| Benzaldehyde..... | 8.59 | 106.05 | 217° | 1.560 | 1.685 | indet. | β |
| Benzylidene acetone.. | 6.89 | 146.08 | 186° | 1.450 | 1.618 | > 1.736 | both |
| Carvone..... | 6.76 | 150.11 | 143° | 1.490 | 1.645 | 1.710 | all |
| Citral..... | 6.70 | 152.12 | 132° | 1.560 | indet. | 1.660 | both |
| Ethyl protocatechuic aldehyde..... | 6.28 | 166.08 | 175° | 1.445 | (1.690) | > 1.736 | 1.690 |
| Heliotropine..... | 6.76 | 150.05 | 234° | 1.580 | indet. | 1.725 | both |
| <i>l</i> -Menthone..... | 6.63 | 154.14 | 184° | 1.528 | indet. | 1.590 | both |
| <i>p</i> -Methyl acetophenone | 7.32 | 134.08 | 210° | 1.445 | indet. | 1.645 | both |
| Methyl-undecyl ketone. | 5.49 | 198.20 | 123° | 1.480 | 1.560 | 1.580 | β |
| β -Thujone..... | 6.70 | 153.13 | 170° | 1.520 | indet. | 1.590 | both |
| Vanillin..... | 6.70 | 152.06 | 230° | 1.692 | indet. | indet. | α |

SUMMARY.

The semicarbazones of six aldehydes and seven ketones found in flavoring materials were prepared and analyzed for purity, and their optical properties were ascertained by the immersion method.

The method given for the identification of these aldehydes and ketones can be applied when only a few centigrams of the substance are available.

The determinative data are given in tabular form for the convenience of the analyst.

Photomicrographs of some of the most characteristic of the semicarbazones are shown.

BOOK REVIEWS.

Bread. A Collection of Popular Papers on Wheat, Flour, and Bread. By HARRY SNYDER, with a biographical sketch by ANDREW L. WINTON. 293 pages. Macmillan Company, New York, 1930. Price \$2.50.

This volume, published after the death of the late Harry Snyder, brings together many of the papers which have appeared under his name in the *Northwestern Miller* during the last quarter of a century. It also contains a number of addresses which he made before conventions of millers, bakers, chemists, grain dealers and food officials.

Professor Snyder was a recognized authority on agricultural chemistry. He was the author of seven books and of more than thirty bulletins published by the United States Department of Agriculture and New York and Minnesota State Experiment Stations, as well as of numerous miscellaneous articles which appeared in scientific and trade journals.

After becoming associated with one of the large wheat milling concerns of the country, Professor Snyder's attention was given for the most part to problems of flour production and utilization. He was a staunch advocate of flour bleaching and a forceful champion of white bread.

The twenty-three chapters in this book are selections from his numerous writings which have more or less bearing on the general theme, although the chapter headings indicate a wide diversity of thought, for example, "Mineral Components of Wheat and Flour and Their Role in Human Nutrition" contrasted with "Some of the Baker's Problems". In spite of the lack of continuity of thought, this book is delightful reading. Professor Snyder had the faculty of expressing his thoughts clearly and in a manner that held the attention of his listeners or readers. In this volume he pays tribute to science for what it does for farm crops and to the "Mill Chemist" for the important place he holds in the manufacture of uniform flour. He describes the improved methods of modern flour milling as well as the primitive methods of the old-fashioned flour mill. He tells of George Washington as a wheat farmer and points out some of the advanced ideas that he held in regard to agriculture. While the book contains much that is scientific and technical, the language is so selected that it is easy to understand. Every one that reads the book will find much of interest relating to this universal food, Bread.

—L. H. BAILEY.

Colloid Symposium Annual (Formerly Colloid Symposium Monograph). Vol. VII. Edited by H. B. Weiser. 296 pages. John Wiley & Sons, Inc., New York, 1930. Price \$4.50.

This volume of the more appropriately entitled "Colloid Symposium Annual" contains the papers presented at the Seventh Symposium on Colloid Chemistry held at Johns Hopkins University, June, 1929. The international atmosphere which had been attendant on previous similar occasions was retained through the interesting presentation of the opening paper on "The Scattering of Light in Sols and Gels", by Prof. F. G. Donnan, the guest of honor. Following Professor Donnan's paper are twenty-two others, representing what can probably be considered the finest American work of the year. A glance at the titles of these papers shows the varied ramifications of colloid chemistry. While a matter of great convenience, it has hardly seemed necessary to list all these papers, but as an aid to our readers, a few of particular interest have been selected as follows:

- The Adsorption of Vapors. Walter A. Patrick.
The Apparent Specific Gravity and Moisture Content of Clay. Frank K. Cameron and Richard A. Lineberry.
The Colloidal Nature of Some Finely Divided Natural Phosphates. K. D. Jacob, W. L. Hill and R. S. Holmes.
The Adsorption of Fats from Volatile Solvents. Harry N. Holmes and Clifford J. B. Thor.
The Chemistry of Bacteria and the Development of a Practical Technique for the Chemical Analysis of Cells. Treat B. Johnson.

Merely a hasty reading of these widely varied papers presents innumerable ideas for future work, but to get the full essence of them will require very careful reading. Even those articles in alien fields may furnish new ideas and a fresh outlook on old problems that have been laid aside.

The papers this past year show a marked advance over those of previous years. Quantitative methods, applicable to colloid chemistry, have been developed, and their use gives a much more detailed picture of colloidal phenomena than that presented by the qualitative work of the past.

It is unfortunate that not more of these articles are summarized. While a summary or conclusion never takes the place of reading an article, it is the ideal short cut for the busy man who desires to keep in touch with the current literature.

For the person who was fortunate enough to hear these papers presented in Baltimore, this book is a wonderful adjunct to an oftentimes faulty memory, while it gives to those who were absent the latest word on the American work in colloid chemistry.

—J. R. ADAMS.

Tanning Materials of the British Empire. Reprinted from the Bulletin of the Imperial Institute, South Kensington, S. W. 7, London, England. Price, two shillings.

The publications of the Imperial Institute of London are always interesting and informative, and the present publication, dealing with the work of the Institute on the tanning materials of the British Empire, is no exception. In fact, it is an excellent summary of existing information on the native vegetable tanning materials which are available in the various portions of the British Empire.

The publication in question contains 100 pages, 3 of which are devoted to an index of Latin and common names of the tanning materials. Thirty-six different tanning materials are described. Detailed information as to localities where grown, tannin content, commercial development, data on exports, etc., are given. There is also a very good bibliography on tanning materials. The publication will be useful to all those directly interested in tanning materials or in leather manufacture.—F. P. VEITCH.

The Condensed Chemical Dictionary. A reference volume for all requiring quick access to a large amount of essential data regarding chemicals and other substances used in manufacturing and laboratory work. Compiled and edited by the Editorial Staff of the Chemical Engineering Catalog. Francis M. Turner, Editor. Second edition. Completely revised and enlarged under the supervision of Thomas C. Gregory, Editor, and Isabelle M. Welch, Assistant Editor. The Chemical Catalog Co., Inc., 1930. 551 pages. Price \$10.00.

This book furnishes information on a great variety of substances used in the chemical, medicinal, engineering and the paint and varnish industries. The facts recorded include place of production, constituents (of mixtures), physical properties, constants, varieties of containers as marketed, grades, uses and railroad shipping regulations. Some topics

like cadmium plating, chromium plating and denatured alcohol are treated at considerable length, so that the information becomes encyclopedic in character. However, most subjects are handled briefly in the usual style of dictionaries. The composition and properties of many substances that are sold under trade names are stated. If a medicinal substance is described in the U. S. Pharmacopeia, National Formulary or New and Nonofficial Remedies, that fact is usually indicated.

Among the numerous useful features in the appendix are specific gravity tables for ethyl and methyl alcohols; metric equivalents, equivalent temperatures in F. and C°, degrees Baumé and equivalent specific gravities, composition of freezing mixtures, boiling points of solutions of various salts of differing strengths, hardness of woods, etc. The second edition is a great improvement over the first, both in the variety and quantity of the facts included and in the care and accuracy with which they have been compiled. The book should prove of great value to salesmen of chemicals, exporters, importers, shippers and plant superintendents. It will not be so valuable to analysts who will wish more detailed information as found in special technical books and journals.—L. E. WARREN.

NEW BOOKS.

Harvey W. Wiley, An Autobiography. The Bobbs-Merrill Company, Indianapolis, Ind., 1930. Price \$5.00. Many of the members of the Association of Official Agricultural Chemists, if not our readers, will turn to this book to refresh their memories of Doctor Wiley's many annual addresses made at the regular session or at the "Wiley Dinner". However this may be, they will continue reading from chapter to chapter because of the intimate details of the personal achievements, the events and incidents in the life of "The Father of the Pure Food Law".

May we be pardoned reference to a personal incident. Upon receipt of our copy for review a friend happened in and took the book up in a more or less skeptical attitude; he first browsed here and there, read a chapter through, and then spent most of the afternoon in thorough enjoyment, reading chapter after chapter. We had to promise the loan of the book at a later date to get him to relinquish it so that we might have it for our own perusal.

The intimate nature of this narrative is revealed in Dr. Wiley's account of his first meeting with Miss Kelton, who became his wife. Because of repairs to the Department Library, Mr. Cutter, the librarian, had been given permission to occupy a room in the Bureau of Chemistry. "After he had moved into the new quarters", says Dr. Wiley, "he came to my office and said: 'We are moved. Come and see the new library'. I went with him upstairs and was just entering the door when I saw a young woman with a book in her hand, apparently looking for the proper place to deposit it. I was immediately struck with her appearance. I seized Mr. Cutter by the arm and said: 'Stop a moment!' I took a second look at this young lady, who was unconscious of being under observation. I turned to my companion and said: 'I intend to marry that girl . . .'. Mr. Cutter replied to my impetuous prediction by saying: 'Perhaps it would be well for you to meet this young lady before proposing matrimony'. A little later he introduced me".

Those of our readers who are unacquainted with the battle surrounding the passing of the Pure Food Laws will find a wealth of material presented here.

Principles of Soil Technology. By PAUL EMERSON. The Macmillan Company, New York City, 1930. Price \$3.25. The author cleverly introduces his subject by the following descriptive paragraph of the soil: "The soil locks within its embrace the be-

ginnings of all life, and receives, at last, their discarded forms. It will outlive all the works of man, transcend all human thought. It traces the progress of history and shelters its ignoble end. It speaks eloquently and is dumb. It is the imperishable storehouse of eternity". The interest that this enticing introduction stimulates in the student is fully sustained in the delightful presentation of the text. The author presents his subject as developed from long experience with advanced students. As he reveals in the preface, this "has convinced him that in order to understand the complex factors governing soil management and soil productivity, it is first necessary that the student understand the principle of the soil as applied to the soil *in situ*. Such principles are just as essential to the equipment of the student of soils as the principles of bodily functions are essential to the medical student. One should know the related factors involved in a manipulation, of the possible results of a correction, before the process is applied". It is only necessary to cite the main subdivisions to indicate the material included in this book. Part I deals with "Soils in General: Their Formation and Classification"; Part II, "The Physical Properties and Functions of Soils"; Part III, "The Chemical Properties and Functions of Soils"; Part IV, "Soil Biology".

NOTICES.

The following circular letter has been sent out by the Referee on Metals in Foods to all chemists who are known to be interested in the Gutzeit method for estimating small quantities of arsenic.

It is hoped that all workers in this field of activity will consider this notice an invitation to attend this symposium, or if this is not possible, to send a letter.

June 20, 1930.

The extensive application of the Gutzeit method for estimating small quantities of arsenic in various agricultural products has led to numerous minor modifications of the official procedure as stated in the *Methods of Analysis* of the Association of Official Agricultural Chemists. For this reason it is proposed to call a general meeting, or symposium, of all chemists who are actively interested in the Gutzeit method at the coming convention of the Association of Official Agricultural Chemists, which will be held in Washington this fall.

You are invited to come and be prepared to present your views on any changes in this important method which you deem advisable to make. If unable to attend, please forward your suggestions in a letter. It is particularly fitting that any desirable changes should be thoroughly discussed at this time, because the 1930 revision of *Methods of Analysis* will be completed late in the fall.

Very truly yours,

G. C. SPENCER,

Referee, Metals in Foods, A. O. A. C.

REFEREES AND ASSOCIATE REFEREES OF THE A. O. A. C.

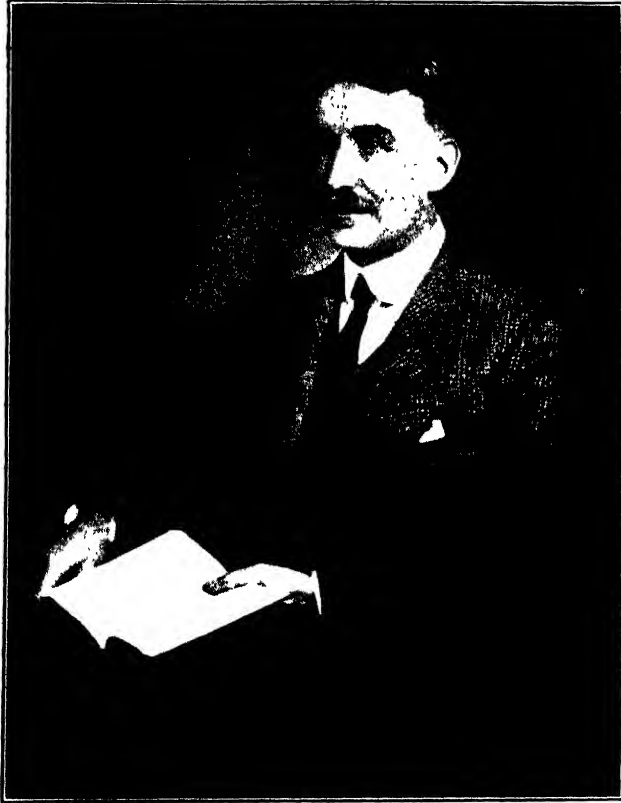
The following circular letter has been sent to all referees and associate referees by the Chairman of the Revision Committee of Methods of Analysis, W. W. Skinner.

As you know, the third revision of the *Book of Methods* is being prepared. The Committee on Revision has followed the recommendations which the association has adopted

during the past six years, both in regard to the incorporation of new material and to the deletion of methods or parts of methods. At the next meeting of the association in October, another and final opportunity will be given to the members to make suggestions regarding changes in the methods. The referees and associate referees naturally are in a position to be of the greatest help to the Committee on Revision, and the committee therefore calls on them to make a thorough study of the methods with which each is most familiar and to report as soon as possible. If it is impossible to do this work immediately, a report may be presented at the meeting in October. All suggestions which relate to actual changes in methods must, of course, meet the approval of the association, but suggestions relating to verbiage and those which will remove ambiguity, simplify the text, and eliminate errors can be incorporated now. Every effort is being made to have this revision of the *Book of Methods* as complete as possible.

Very truly yours,

W. W. SKINNER.



WYATT WILLIAM RANDALL, 1867-1930

WYATT WILLIAM RANDALL

Wyatt William Randall was born in the historic city of Annapolis, Maryland, on January 10, 1867. He died suddenly after a minor operation on the morning of July 22nd last, in the city of Baltimore, where he had lived since 1910.

After graduation from St. John's College, Annapolis, in 1884, Dr. Randall entered the graduate school of chemistry at Johns Hopkins University, where he received his Ph.D. in 1890. From 1890 to 1898 he taught at Johns Hopkins, with the rank of assistant and later associate in chemistry. During the two following years he was science master at the Lawrenceville School, New Jersey, and the next year he was professor of chemistry at Tome Institute, Port Deposit, Maryland. From 1901 to 1910 he was Head Master of the Mackensie School at Dobbs Ferry, New York. In 1910 he became associated with the Maryland State Department of Health, which connection was continued until his death. He was appointed chief of the Bureau of Chemistry of that organization in 1916.

In February, 1918, Dr. Randall began his work as a member of the Joint Committee on Definitions and Standards of the U. S. Department of Agriculture, from which he resigned on February 16, 1926. In 1922 he was President of the Central Atlantic States Dairy, Food and Drug Officials, an association which he had helped to organize.

Dr. Randall's contact with the Association of Official Agricultural Chemists began with the November, 1913, meeting, and, with but one exception, he attended every meeting until his death. During this period of eighteen years he served the Association in various offices, such as General Referee on Preservatives, member of the Board of Editors, member of the Committee on Recommendations of Referees, and member of the Executive Committee, this activity culminating in his election to the presidency at the 1925 meeting. At the time of his death he was a member of the important Committee on Editing Methods of Analysis.

Dr. Randall's professional career, covering a period of forty-six years, is one which all may seek to emulate. Whole-hearted devotion to the science of chemistry and the application of chemistry to the cause of purity in foods and drugs were his life work. The fascination of a career in science, its rewards and the public aspect of his official duties outweighed all considerations of commercial success. To his chosen field of work he brought an unusually effective mental equipment, and the impressive degree of accuracy he attained in his experimental work was a reminder of those earlier days of association with Remsen and Morse.

To see a piece of laboratory apparatus set up by Dr. Randall was a lesson which would be useful to many chemists of the present day, and this same workmanship and precision was carried throughout his actual analytical and experimental details. Even his notebooks, which were models of clarity of expression and thorough observation of experimental phenomena, were written with the same meticulous neatness and attention to detail which characterized all his work. This attention to detail, however, did not lessen his enthusiasm and the amount of work he accomplished.

Dr. Randall always impressed his associates with his devotion to the fundamental principles of his chosen field of endeavor and to his high ideals. His ethics were of the highest standard. In his capacity as director of the Bureau of Chemistry of the Maryland State Department of Health, Dr. Randall was beloved by all his fellow workers. He was never too busy to undertake a new line of work and was always ready to give to his subordinates the benefit of his broad training and rich experience. His long connection with and his ripe experience in the legal aspects of the food and drug work gave him an exceptional background for the development and training of men for that particular field. His personality, which was ever gentle and considerate of the feelings and opinions of others, his perfect command of the English language and his wide interests and experiences based on extensive travel both in this country and abroad gave him a cultural foundation which immediately commanded the attention of all who met him.

No summary of Dr. Randall's life and work would be complete without mention being made of the extremely valuable services which he rendered as a member of the Joint Committee on Definitions and Standards. In one of the most difficult and important phases of this work—the phrasing of the definitions in such terms as to make them absolutely clear and capable of but a single interpretation—Dr. Randall was the recognized authority, and those of us who had the pleasure of being associated with him in this connection will always recall the infinite patience with which he dealt with this problem.

Those who knew Dr. Randall will ever cherish his memory as that of a man thoroughly trained in science and devoted to his ideals and work, a true friend and adviser, a man of splendid judgment, an inspiring teacher, a gentleman of the old school whose culture was outstanding but never obtrusive, and a man whose promise to undertake a given assignment was an assurance of its accomplishment—in other words, a man who always followed the line of duty and responsibility with never a thought of failure.

His participation in all the work of the Association of Official Agricultural Chemists was active and hearty; rarely are men found with Dr. Randall's equipment who will give so constantly and generously of their time and experience in the development of any organization. His passing is a real loss to the association and to food chemistry in America. May others be stimulated by Dr. Randall's career and activities to those goals of science and public service which he so eminently achieved.

F. C. BLANCK.

EDITORIAL.

THE VALUE OF ATTENDING MEETINGS.

Probably there is little necessity for urging the chemist personally to attend the meetings of the Association, nor is there any call to elaborate upon the benefits to be derived from his attending all meetings of scientific societies. However, it is desirable to direct attention to the value of attending the meetings of those organizations that are likely to provide sources of information in particular fields of chemistry.

The opportunity to meet fellow workers occasionally and to discuss with them their respective problems frequently hastens the completion of one's own research. It is with this idea in mind that attention is directed to the following incident, which so aptly illustrates our point.

In 1920 Hoffer and Carr¹ published their work relating to the effect of introducing solutions of iron, copper and aluminum compounds into the internodal cortical tissues of corn stalks. They showed that the effects, in some instances, were similar to those observed in stalks affected by root rot.

At the meeting of the Hawaiian Section of the American Chemical Society, held in Honolulu, November 1, 1924, some five years after publication of Hoffer and Carr's work, McGeorge² presented his studies of toxic conditions associated with the root rot problem presented in connection with sugar cane. Now, we wish to stress the fact that McGeorge was enabled to discover the similarity of his problem to that of the former workers by reason of a visit to a "number of Agricultural institutions on the main land", as he puts it, where he personally met Dr. Hoffer.

While it is true that publication of other results by Hoffer and Carr³ had appeared, yet a chance contact and meeting with one of the authors gave McGeorge the opportunity of discussing their problems.

The benefit of this meeting extended to others, if we follow up what occurred later. Earle⁴, who for twenty years had worked with sugar cane problems, says: "I wish to thank Mr. W. T. McGeorge for his important article on the 'Root Rot Problem of Sugar Cane * * *'. From it I have learned for the first time of the accumulation of iron and aluminum in the vascular systems of the nodes of diseased stalks and the fact that potash fertilizers may in some cases prevent these accumulations".

It will be helpful to those in administrative positions, who may at times require material evidence of the value of our meetings, if those most interested and in a position to provide specific incidents will stress them and play them up—in other words, "sell the idea" to them.

¹ *Phytopathology*, 10, 57 (1920).

² *Facts About Sugar*, 20, 730 (1925).

³ *J. Agr. Research*, 23, 801 (1923).

⁴ *Facts About Sugar*, 20, 882 (1925).

SECOND DAY.
TUESDAY—MORNING SESSION.

REPORT ON EGGS AND EGG PRODUCTS.

By SAMUEL ALFEND (U. S. Food and Drug Administration, St. Louis, Mo.), *Referee*.

The referee undertook to carry out the recommendations¹ on ash, unsaponifiable matter, and alcohol-precipitable water-soluble protein. The collaborators, all from the U. S. Food and Drug Administration, St. Louis, reported ash results on liquid whole egg, liquid yolk, and powdered dried whole egg by the tentative method². The results are given in Table 1. Considering the nature of the product and the difficulty of ashing, the agreement among analysts' results is considered quite satisfactory, although there is a maximum variation of 0.24 per cent in the results on powdered dried egg. The results are somewhat better than last year's.

Only one analyst's results on unsaponifiable matter are available, and these do not check values obtained by the referee on one of these samples. This work should be continued during the next year.

The referee devoted considerable attention to the method for water-soluble protein precipitable by 40 per cent alcohol. It was found that the method cannot be relied upon to give concordant results on the same sample at different times, or to yield the same kind of precipitate and of solution on two samples of egg run side by side. The cause of the physical differences, such as the formation of a fairly clear aqueous solution of the proteins in one sample and an almost milky, colloidal suspension in another, or of a flocculent alcohol precipitate at one time and a colloidal solution at another, has not been worked out satisfactorily. Undoubtedly one source of error is the frequent presence in the aqueous solution of a very fine suspension which comes through the filter mat, and which is frequently carried down in the subsequent alcohol precipitate, causing high results. Another source of error is the failure of the precipitate to coagulate, leading to a loss of nitrogen by the passage of the fine precipitate or colloidal suspension through the filter pad.

There are numerous manipulative difficulties in the method. One of these, the violent bumping during the Kjeldahl digestion due to the presence of asbestos, may be readily overcome by the addition of glass beads. Another one, the occasional difficulty in getting the alcohol precipitate to pack well on centrifugalizing, leads to a slowing up of

¹ *This Journal*, 12, 80 (1929).

² *Ibid.*, 55.

the filtering process, sometimes with a loss of the precipitate through the filter pad. Much time is consumed in centrifugalizing the precipitate.

An attempt was made to aid the packing of the alcohol precipitate by the addition of powdered calcium carbonate, after it was apparent that asbestos did not do this. The calcium carbonate, like asbestos, separated from the precipitate during the centrifugalization and proved to be valueless. Alumina cream was found to carry down the precipitate completely, in a short time, leaving a clear solution which could be poured off without the necessity of filtering it through asbestos. The results on aliquots treated with alumina cream were considerably higher than those on the aliquots not so treated. Part of this difference was due, probably, to the better packing of the precipitate, so that no precipitated protein was lost, and part was due, in some cases at least, to the carrying down of finely suspended matter which came through the filter mat in the aqueous extraction. Possibly some error was caused by the absorption by the alumina cream of alcohol-soluble protein which the subsequent washing failed to remove.

The following procedure was carried out to determine whether the high results with alumina cream were due to the carrying down of particles which should have been filtered out of the aqueous extract. Duplicate samples of various egg products (powdered whole egg, flake dried egg, and liquid egg), which had been found to give cloudy aqueous extracts, were extracted with water, and one determination of each sample was treated with 5 cc. of alumina cream before centrifugalizing and filtering.

The clarification was excellent, every solution treated with alumina cream filtering through almost sparkling clear. Aliquots of clarified solutions which were treated with alumina cream in the subsequent precipitation with alcohol gave higher results than those determined without alumina cream, but the differences were not nearly so marked as they were in the unclarified solutions. All the determinations on the clarified aqueous extracts gave considerably lower values for nitrogen than were obtained on the duplicate solutions that had not been clarified. These results lead to the suspicion that perhaps the alumina cream carried down colloidal albumin in the aqueous extracts.

The work last year indicated that sodium chloride does not play the same rôle in eggs that it does in flour and noodles. This year's investigation disclosed that aliquots of egg extracts precipitated in the absence of salt gave distinctly lower results, and in several cases failed to give a precipitate. With flour and noodles, on the other hand, the precipitation in the absence of salt gives results as high as or higher than those obtained by precipitation from a 1.2 per cent salt solution. Approximately the same results were obtained whether the salt was added before or after the addition of alcohol. It was found necessary to allow 12-18

hours for the coagulation of the precipitate in the presence of salt, and longer when no salt was added.

Palmer¹ and the referee have used 1.2 per cent sodium chloride solution to extract the soluble proteins from egg noodles in order to obtain a clear solution, and collaborative results by this method were excellent². However, the results were higher than those obtained from an aqueous extract, such as was used in the tentative methods for flour and eggs. Consequently, the use of salt solution for extraction was discontinued.

It is believed that future work should include a study of extraction of egg products, and of egg noodles and flour as well, with a 1.2 per cent sodium chloride solution, and the use of alumina cream for clarification and for subsequent precipitation. The adoption of such modifications may make it necessary to discard some authentic data, and it may nullify the value of some of the excellent work of Buchanan³. The data on water-soluble protein in egg products assembled by Hertwig⁴ and Buchanan are extremely meager and not particularly concordant, and it should not be difficult to revise their ratios for differentiating between whole egg and egg yolk products.

No associate referee was appointed on total solids, fat, lipoids and lipid P_2O_5 , but the referee was able to familiarize himself with the fat and lipid methods, and to study the methods for total solids collaboratively. The results are given in Table 2.

Previous referees have determined that the vacuum oven method at 98°C. gives true results for total solids, that analysts are able to obtain closely concordant values, and that the more rapid air-drying method gives results in fair agreement with those obtained by the vacuum method.

This year's study, during which all the analysts worked on the same samples, demonstrates that close agreement is obtainable by different analysts by the vacuum oven method on liquid whole egg, liquid egg yolk, and powdered dried whole egg. The results by the rapid air oven method show a greater variation, but the average values check closely with those obtained by the vacuum oven method, except in the case of the powdered dried egg, in which the air drying yields uniformly high results.

No report was submitted by the Associate Referee on Detection of Decomposition.

RECOMMENDATIONS⁵.

It is recommended—

(1) That methods for the determination of fat (acid hydrolysis), lipoids and lipid phosphoric acid (P_2O_5) be studied collaboratively.

¹ *This Journal*, 8, 615 (1925).

² *Ibid.*, 11, 490 (1928).

³ *Ibid.*, 7, 407 (1924).

⁴ *Ibid.*, 88 (1923).

⁵ For report of Subcommittee C and action of the association, see *This Journal*, 13, 70 (1930).

(2) That methods for the determination of total phosphoric acid (P_2O_5), with consideration of the use of potassium hydroxide and magnesium acetate or nitrate as fixing agents, be studied.

(3) That methods for the determination of added sugars be studied.

(4) That the tentative 98°C. vacuum oven method¹ be adopted as official (first action).

(5) That the tentative 112°–117°C. air oven method² be further studied collaboratively with a view to its adoption as an official method.

(6) That the tentative method for the determination of ash² be adopted as official (first action).

(7) That the tentative method for the determination of unsaponifiable matter³ be studied collaboratively with a view to its adoption as an official method.

(8) That the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in egg products be further studied in conjunction with the same methods for alimentary pastes and flour, with consideration of the use of 1.2 per cent sodium chloride solution for extracting the sample and of alumina cream for clarifying, this project to be accompanied by collaborative work if possible.

(9) That the method for the determination of acid-soluble phosphoric acid (P_2O_5) be further studied, and be accompanied by collaborative work.

(10) That a study be made of methods for determining ammonia nitrogen and reducing substances as dextrose.

TABLE 1.

Collaborative results on ash in eggs.

| ANALYST | LIQUID EGG | LIQUID YOLK | POWDERED DRIED WHOLE EGG |
|--------------------------------|-----------------|-----------------|-----------------------------|
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| R. S. Pruitt | 1.17 | 1.79 | 4.07 |
| | 1.17 | 1.84 | 4.14 |
| J. P. Aumer | | 1.70 | 3.90 |
| | | 1.76 | 3.97 |
| | | 1.76 | |
| | | 1.78 | |
| Samuel Alfend | 1.16 | 1.72 | 3.93 |
| | 1.17 | 1.74 | 3.98 |
| Average | 1.17 | 1.76 | 4.00 |
| Variation | 0.01 | 0.12 | 0.24 |
| Average deviation from average | 0.00 | 0.03 | 0.07 |

¹ *This Journal*, 9, 56 (1926).

² *Ibid.*, 12, 55 (1929).

³ *Ibid.*, 56.

TABLE 2.

Collaborative results on total solids in eggs.

| ANALYST | LIQUID EGG | | LIQUID YOLK | | POWDERED DRIED WHOLE EGG | |
|-----------------------------------|-------------------|-----------------------|-------------------|-----------------------|-----------------------------|-----------------------|
| | Drying at | | Drying at | | Drying at | |
| | 98°C. in vacuo | 112°-117°C. in air | 98°C. in vacuo | 112°-117°C. in air | 98°C. in vacuo | 112°-117°C. in air |
| | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| R. S. Pruitt..... | 26.78 | 26.76 | 49.09 | 49.06 | 92.78 | 93.03 |
| | 26.82 | 26.80 | 49.13 | 49.18 | 92.88 | 93.10 |
| J. P. Aumer | 26.67 | 26.75 | 49.15 | 49.34 | 92.77 | 93.06 |
| | 26.69 | 26.86 | 49.22 | 49.41 | 92.81 | 93.15 |
| Samuel Alfend | 26.69 | 26.57 | 49.10 | 48.75 | 92.94 | 93.03 |
| | 26.73 | 26.63 | 49.19 | 48.97 | 92.94 | 93.06 |
| Average | 26.73 | 26.73 | 49.15 | 49.12 | 92.85 | 93.07 |
| Variation | 0.15 | 0.29 | 0.13 | 0.66 | 0.17 | 0.12 |
| Average deviation from average | 0.05 | 0.09 | 0.04 | 0.19 | 0.07 | 0.04 |

Water-soluble protein, unsaponifiable matter and ash. See report by Referee on Eggs and Egg Products.

No report on detection of decomposition was given by the associate referee.

No referee on total solids, fat, lipoids, lipid P_2O_5 was appointed. See report by the Referee on Eggs and Egg Products.

REPORT ON PRESERVATIVES.

By WYATT W. RANDALL* (State of Maryland Department of Health, Baltimore, Md.), *Referee*.

During the year 1927, G. W. Monier-Williams published an extended report¹ dealing with such questions as: (1) the nature of the compounds formed through the interaction of certain sugars and other aldehydic and ketonic substances with sulfurous acid and its salts; (2) the dissociation of such compounds through the action of hot dilute hydrochloric acid; (3) the action of a cold solution of hydrogen peroxide upon sulfur dioxide, as compared with its action upon other volatile sulfur

* Deceased.

¹ Reports on Public Health and Medical Subjects, No. 43: London: The Ministry of Health. Cf. *Brit. Food J.*, 29, 51-3 (1927).

compounds; and, as a result of these studies, (4) the development of an improved method for the determination, in foodstuffs, of added sulfurous acid or sulfites.

The proposed method, while retaining many of the characteristics of the official method, differs in certain details. It makes use of an efficient reflux condenser attached to the boiling-flask; this effects the return to the flask of the vapors of hydrochloric acid or of volatile organic acids, while permitting sulfur dioxide to pass over into the receiver. The substitution of hydrochloric acid for phosphoric effects a more rapid decomposition of such sulfite compounds as may exist in the sample under examination. The selective action of cold, dilute hydrogen peroxide solution results in the complete oxidation of any sulfur dioxide that may come in contact with it, without producing any effect upon hydrogen sulfide or upon any volatile organic sulfur compounds that may escape condensation and so may be swept into the receiver. Consequently, since the only free acid present in the receiver at the end of the distillation is the sulfuric acid formed by oxidation of sulfur dioxide, its amount, and hence that of the sulfurous acid from which it was produced, may be found by titration with a standard solution of alkali. Furthermore, a gravimetric determination of the sulfuric acid formed may be carried out as a substitute for, or in addition to, the volumetric.

Monier-Williams found that when hydrochloric acid is employed instead of phosphoric, preliminary treatment of the sample with hot sodium carbonate solution is unnecessary; all the sulfur dioxide is evolved and with no great delay.

In the list of foodstuffs which Monier-Williams examined by the proposed method are the following: Cornmeal, port wine, dried apricots, fresh nectarines, sultanas, gelatin, sausage, corn sirup, sugar, jam, mustard, and onions. Many of the same samples were examined by other analysts employing other methods; the agreement among the results is remarkably close—whether volumetric or gravimetric—whether with or without preliminary treatment with sodium carbonate. The point seems to be established, in the case of dried fruits, that prolonged boiling with dilute hydrochloric acid is essential if complete separation of the sulfur dioxide from the sample is to be accomplished; from one hour to two hours and a half is often necessary. When phosphoric acid was used the evolution of gas was even slower.

A study of the results given in this paper leads to the conclusion that superiority of the Monier-Williams method over the official method is manifest in a number of ways. First, and of chief importance, it is capable of determining accurately the amount of sulfite present even in such materials as onions and mustard, in spite of the presence of considerable quantities of volatile organic sulfur compounds; whereas, in the official method, such compounds are oxidized by bromine, and the

sulfuric acid formed from them is not to be distinguished from that which had its source in sulfite. Second, it makes possible the employment of a titration method for the estimation of the sulfur dioxide which has been set free from the sample under examination, while at the same time permitting the use of the gravimetric determination as well. Third, it operates without causing frothing of the heated sample. Fourth, the sulfur dioxide is set free more quickly and more completely. Fifth, it does away with the use of bromine.

In the first number of *The Journal* for the current year¹, J. Fitelson published the results of an investigation whose purpose was to compare, from the standpoint of accuracy, the Monier-Williams method and the present official method for the determination of sulfur dioxide present, in one form or another, in food products. Fitelson finds the proposed method in no case inferior, and in many cases he finds it far superior, to the present official method. The gravimetric estimation he finds somewhat more accurate than the volumetric, especially if such quantities of sulfur dioxide as 1-4 mg. are to be determined. In a series of tests applied to brown mustard seed, the results showed that the sulfur dioxide added was recovered with an error of only 1-3.5 per cent of its amount. The most striking facts brought out in Fitelson's paper, however, are in connection with estimations of sulfur dioxide added to brined onions. Except in one case, where the amount added was less than 3 mg., the error was in no case over 2 per cent of the amount to be estimated. On the other hand, the results obtained with the aid of the official method ranged up to 10 or 20 times the amount of sulfur dioxide actually present.

The hydrogen peroxide solution employed by Fitelson was prepared by treating a 30 per cent solution, after diluting somewhat, with barium hydroxide solution until exactly neutral in the presence of bromphenol blue. After settling has taken place, the solution is filtered, its strength is determined by titration with permanganate solution, and its concentration is reduced by dilution to about 3 per cent.

The referee hoped to be able in the interval between his receipt of Monier-Williams' paper and this meeting of the association to subject the new method to cooperative study. This has not been possible. However, the appearance of Fitelson's paper, with its thoroughgoing support of all that has been claimed for the Monier-Williams method and the further evidence of the failure of the official method to measure up to its work, leads the referee to believe that the matter should receive prompt attention. Accordingly he offers the following recommendations²:

¹ *This Journal*, 12, 120-129 (1929).

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 71 (1930).

RECOMMENDATIONS.

(1) That the Monier-Williams method for the estimation of added sulfurous acid or added sulfite in food products, as described in Fitelson's paper, be adopted as a tentative method.

(2) That during the coming year the Monier-Williams method be studied cooperatively with a view to its substitution a year hence for the present official method¹.

REPORT ON COLORING MATTERS IN FOODS.

By C. F. JABLONSKI (U. S. Food and Drug Administration, New York, N. Y.), *Referee*.

Following the recommendation of the association, the referee sent four samples of mixtures containing amaranth and tartrazine to five collaborators, with a request to estimate the dyes by the method submitted by the referee at the last meeting. Unfortunately, only three reports were received.

The compositions of the mixtures (based upon titanium trichloride titrations) were as follows:

| NO. | AMARANTH <i>per cent</i> | TARTRAZINE <i>per cent</i> | TOTAL DYE |
|-----|-----------------------------|-------------------------------|-----------|
| 1 | 45.0 | 45.8 | 90.8 |
| 2 | 22.5 | 67.7 | 90.2 |
| 3 | 67.5 | 22.9 | 90.4 |
| 4 | 22.5 | 22.9 | 45.4 |

The following results were submitted by the collaborators:

| | NO. | AMARANTH <i>per cent</i> | TARTRAZINE <i>per cent</i> | TOTAL DYE <i>per cent</i> |
|------------------|-----|-----------------------------|-------------------------------|------------------------------|
| O. L. Evenson | 1 | 46.3 | 40.0 | 86.3 |
| | 2 | 26.0 | 66.4 | 93.3 |
| | | 27.2 | 67.0 | |
| | 3 | 67.0 | 18.6 | 85.55 |
| | | 66.2 | 19.3 | |
| | 4 | 25.5 | 20.0 | 45.75 |
| J. T. Bashour | | 24.0 | 22.0 | |
| | 1 | 40.13 | 45.10 | 85.22 |
| | | 40.05 | 45.15 | |
| | 2 | 20.07 | 68.79 | 89.25 |
| | | 20.95 | 68.70 | |
| | 3 | 58.25 | 26.89 | 85.04 |
| W. C. Bainbridge | | 58.29 | 26.66 | |
| | 4 | 19.56 | 23.14 | 42.67 |
| | | 19.39 | 23.25 | |
| | 1 | 40.8 | 39.9 | 80.35 |
| | | 39.9 | 40.1 | |
| | 2 | 14.9 | 66.3 | 81.35 |
| | | 14.9 | 66.6 | |
| | 3 | 56.3 | 21.9 | 77.70 |
| | | 55.1 | 22.1 | |
| | 4 | 18.1 | 21.1 | 39.4 |
| | | 18.1 | 21.5 | |

¹ *Methods of Analysis*, A. O. A. C., 1925, 135.

The following comments and criticisms were submitted by the collaborators:

O. L. Evenson.—The results appear to be somewhat low, except in case of No. 2. The titration of small amounts of dye would tend to give high results, and this compensates to some extent for possible losses sustained elsewhere. It seems that it should not be necessary to shake the funnels for 2 minutes. Continuous washing with N/128 hydrochloric acid and water changes considerably the proportion of gasolene and amyl alcohol.

J. T. Bashour.—(1) In determination of mixture No. 1, the reduction of one aliquot was made by standing overnight instead of heating to 50°–60°C. for 3 hours. The results are not noticeably different from the other determinations, which were made strictly according to method. (2) Two minutes of vigorous shaking is not necessary to extract the mixed dyes with amyl alcohol. (3) After the addition of petroleum ether, persistent emulsions usually were formed on vigorous shaking and cleared up only on centrifuging. This trouble was entirely eliminated in later determinations by simply shaking the funnels more gently. (4) The volume of amyl alcohol-petroleum ether mixture in the first funnel tends to decrease on continued extraction. This can partly be eliminated by saturating the N/4 hydrochloric acid with amyl alcohol and the N/128 hydrochloric acid with the amyl alcohol-petroleum ether mixture.

W. C. Bainbridge.—Insofar as the method itself is concerned, the only criticism is that the addition of 3 cc. of ammonia solution after reduction is often insufficient, and while the sample may be alkaline to litmus immediately after addition of the ammonia, it may and quite often does become acid to litmus after centrifuging. As failure to make the solution completely alkaline makes a large error, it is recommended that 4 cc. of ammonia be added instead.

Although the results submitted by the collaborators are by no means accurate, nevertheless it is encouraging to note that many are closely so, and it is therefore the belief of the referee that with more experience with the technic of the method better results may be expected. In justification of this statement, attention may be called to the figures given below, which were obtained by adding the percentage composition of amaranth and of tartrazine in the four mixtures and comparing these figures with those reported by the collaborators. The actual composition of the standard was 157.5 per cent of amaranth and 159.3 per cent of tartrazine.

| | TOTAL AMARANTH | TOTAL TARTRAZINE | TOTAL DYE | ERROR FOR TOTAL DYE |
|------------|-------------------|---------------------|-----------|------------------------|
| | <i>per cent</i> | <i>per cent</i> | | <i>per cent</i> |
| Evenson | 164 2 | 146.6 | 310 8 | — 1 9 |
| Bashour | 138 3 | 163 9 | 302 2 | — 4 7 |
| Bainbridge | 129 05 | 149 75 | 278 8 | —12 0 |

The referee suggests that additional collaborative work be undertaken on this problem, and that those suggestions of the collaborators be adopted which will meet the approval of the referee.

Some progress can be reported in connection with the problem of separating amaranth from tartrazine in the presence of indigo carmine as well as sucrose, but owing to incomplete experimental data a report must be postponed for a later date.

In connection with the problem of separation and quantitative estimation of fast green FCF from other permitted coal tar dyes, the referee desires to state that this report is incomplete. However, a brief résumé of the results obtained is herewith submitted.

Fast green FCF, light green SF yellowish and guinea green B belong to the triphenyl methane type of dyes and as such are soluble in α -dichlorohydrine. However, fast green FCF and light green SF yellowish are not extracted to any large extent by amyl alcohol even from normal hydrochloric acid solution. Guinea green B, on the other hand, is almost completely extracted by amyl alcohol from a 5 per cent salt solution or from a weak acid (N/16 hydrochloric). This property is utilized in separating guinea green B from the other two triphenyl methane greens.

To separate fast green FCF from light green SF yellowish, cognizance was taken of the fact that fast green FCF reacts with bromine with the formation of a blue solution, while light green SF yellowish, under similar treatment, is destroyed¹.

The procedure was conducted as follows:

To 25 cc. of 1 per cent fast green FCF solution in a 250 cc. beaker, add sufficient water to make a volume of approximately 70 cc., and after acidifying with 4 cc. of concentrated hydrochloric acid, heat the solution to boiling and gradually add 25 cc. of a solution of 0.1 N bromide-bromate from a pipet, stirring vigorously. Continue the boiling for 5 minutes, remove the beaker from the flame, and place over a steam bath; evaporate the contents to about $\frac{1}{2}$ of the original volume. After an addition of 5 cc. of strong ammonia water (to prevent carbonization), evaporate the contents to dryness, and later transfer with water to a 250 cc. volumetric flask.

The changes occurring during these operations are the following: The addition of hydrochloric acid produced a greenish solution, which was changed by the bromide-bromate at first to a brownish yellow, and later to a deep blue color, while ammonia made a purplish solution. The solubility of the resulting color solution in amyl alcohol was materially altered, as it was almost completely extracted from a N/16 hydrochloric acid.

Wool dyed from a slightly acid solution of the dye, with a deep blue black shade, which was stripped and redyed with a reddish black.

Spot reactions of dyed wool:

| | |
|--|---------------|
| Concentrated hydrochloric acid | light green |
| Concentrated sulfuric acid | brown |
| 10 per cent sodium hydroxide | reddish brown |
| Concentrated ammonia | violet blue |

Reaction of the aqueous solution:

| | |
|--|---------------|
| With addition of small amount of water | blue black |
| With addition of large amount of water | reddish blue |
| Hydrochloric acid to aqueous solution | greenish blue |
| Acetic acid to aqueous solution | blue |
| Strong ammonia to aqueous solution | bluish red |
| 10% sodium hydroxide to aqueous solution | orange brown |

¹ *This Journal*, 12, 354 (1929).

A solution of 25 cc. of 1 per cent light green SF yellowish, treated similarly, produced a brownish yellow coloration.

To test the reliability of the method, a series of mixtures containing fast green FCF and light green SF yellowish was prepared. A 1 per cent stock solution of the dyes was titrated with standard titanium trichloride. The following values were obtained:

| | <i>per cent</i> |
|-------------------------------|-----------------|
| Fast green FCF..... | 85.8 |
| Light green SF yellowish..... | 83.3 |

From the above dye solution the following mixtures were prepared and treated as described above.

Fast green FCF and light green SF yellowish

| | <i>cc.</i> | <i>gram</i> | <i>cc.</i> | <i>gram</i> |
|----|------------|-------------|------------|-------------|
| 1 | 0.0 | | 25.0 | 0.2083 |
| 2 | 0.1 | 0.0009 | 24.9 | 0.2074 |
| 3 | 0.2 | 0.0017 | 24.8 | 0.2065 |
| 4 | 0.5 | 0.0043 | 24.5 | 0.2041 |
| 5 | 1.0 | 0.0086 | 24.0 | 0.1999 |
| 6 | 2.0 | 0.0172 | 23.0 | 0.1916 |
| 7 | 5.0 | 0.0429 | 20.0 | 0.1666 |
| 8 | 10.0 | 0.0859 | 15.0 | 0.1250 |
| 9 | 15.0 | 0.1285 | 10.0 | 0.0833 |
| 10 | 20.0 | 0.1716 | 5.0 | 0.0417 |
| 11 | 25.0 | 0.2146 | 0.0 | |

The results obtained were the following: From Nos. 1 to 3, inclusive, brownish solution; No. 4, a brownish solution with a distinctive redder shade than No. 3; Nos. 5 and 6, purplish solution. Nos. 7 to 11, deep blue solutions progressively increasing in intensity. It was considered advisable to examine solutions Nos. 1 to 4, inclusive, to determine whether any coloring matter could be isolated. To that end these solutions were acidified with hydrochloric acid and extracted individually with two 50 cc. portions of amyl alcohol. The solvent was repeatedly washed with water until no more coloring matter was extracted.

After the addition of an equal volume of petroleum ether, the color was removed with several 25 cc. portions of 5 per cent ammonia water (5 + 95) until no more color was extracted. These were combined and evaporated to dryness and later dissolved in water and transferred to 100 cc. volumetric flasks. The following results were noted: No. 1, a light yellow solution; Nos. 2, 3, and 4, bluish red solutions with increasing intensity.

These experiments would indicate that 4 per cent of fast green FCF can readily be detected in mixtures with light green SF yellowish, while quantities as low as 0.4 per cent of fast green FCF can be determined only after extracting with amyl alcohol, removing the decomposition

products, diluting with petroleum ether, and extracting the color by means of dilute ammonia. Efforts to estimate the color by means of standard titanium trichloride were not entirely successful; therefore additional investigational work is necessary.

RECOMMENDATIONS¹.

It is recommended—

(1) That additional samples of mixtures of amaranth and tartrazine be sent to various collaborators.

(2) That the problem of quantitative separation and estimation of fast green FCF from light green SF yellowish be continued.

(3) That work be undertaken to separate the recently adopted dyes (sunset yellow, ponceau SX and brilliant blue FCF) from the other permitted colors.

REPORT ON METALS IN FOODS.

By G. C. SPENCER (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

The study of metals in foods and feeding stuffs in this association has hitherto been confined primarily to the detection and estimation of such metals as were known or believed to exert a harmful or deleterious effect on the health of the consumers of such foods. This phase of the work, however important it may be in itself, should be supplemented by a consideration of the general prevalence of many metallic elements in plant and animal tissues. This was the general theme of President Schreiner's address to this association in 1928², and the importance of the subject has been recognized by the appointment of associate referees on less common metals in soils and plants.

The regulatory work of the government service requires the most accurate methods possible for detecting any harmful substances that may be present in foodstuffs in amounts sufficient to endanger the public health. The investigational branch is equally desirous of learning what constitutes a properly balanced diet for human beings and animals.

During the past year associate referees have been appointed to study the present methods of the association for the determination of arsenic, boron, tin and copper.

The Associate Referee on Arsenic was asked to study the volumetric methods which have been under consideration in the Western District of the Food and Drug Administration for some time. The Associate Referees on Boron, Copper, and Tin were asked to make a thorough

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 72 (1930).

² *This Journal*, 12, 16 (1929).

survey of the present published methods for their respective metals and if necessary to seek better methods by a comprehensive survey of chemical literature.

RECOMMENDATIONS¹.

It is recommended—

(1) That the work on volumetric methods for the determination of arsenic be studied with collaboration if possible.

(2) That the work already undertaken on boron, copper, and tin be continued.

(3) That the work be continued on methods for the determination of lead, especially as applicable to the determination of this element in spray residue.

(4) That the work on zinc be postponed.

REPORT ON ARSENIC IN FOODS.

By W. C. TABER² (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*.

The more or less general dissatisfaction with the Gutzeit method for the determination of arsenic has led this laboratory to investigate a possible substitute for it. H. R. Smith and others have adapted the bromate method used in insecticides and drugs to the determination of arsenic in foods, particularly sprayed fruit. This method involves the preliminary destruction of the organic matter by acids, and the subsequent distillation of arsenic as arsenic trichloride and its titration with potassium bromate. It has the advantage of a short period of distillation, but the disadvantage of a preliminary acid digestion. It has also the disadvantage of a smaller titration for the same amount of arsenic as compared with the method given in this report. The general procedure of the outlined method was suggested by C. R. Smith³. It is applicable to larger amounts of arsenic than can be accurately found by the Gutzeit method, being capable of determining amounts from 0.2 to 10 mg. Apparently this suggestion was not followed up by other chemists, as the need of the determination of arsenic within the range stated was not pressing; the Gutzeit method was available for the smaller amounts, and the standard insecticide methods for larger ones. Within recent years, however, the determination of arsenic in the spray residue found on fruits has become very important, and the development of a satisfactory method that may be used in the field has engaged the attention of a number of workers. Efforts have been made in this laboratory

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 72 (1930).

² Presented by G. C. Spencer.

³ U. S. Dept. Agr. Bur. Chem. Circ. 102 (1912).

to evolve a method that might be used in the field by working directly on the solution obtained by washing the fruit with from 300 to 500 cc. of acid or alkali, according to which was desired.

In the application of the Smith procedure, essential modification was necessary to meet the different conditions. In the first place, the large volume of solution used compelled the adoption of a large generator flask and also the application of sufficient heat to secure the proper reduction and a vigorous ebullition of gas from the larger volume of liquid, the heat of the reaction being insufficient. The use of the condenser was also deemed expedient, because by this means the distillation could be run much faster, with no danger of carrying over steam and contamination from the lead acetate cotton.

PROPOSED METHOD.

The method is based upon the fact that when arsine is passed into a mercuric chloride solution, there may be formed arsenides and other arsenic and mercury compounds that are titrated with an iodine solution. Whatever compounds are formed, the reaction with iodine is essentially a titration from AsH_3 to As_2O_5 , according to the equation: $2\text{AsH}_3 + 16\text{I} + 5\text{H}_2\text{O} = \text{As}_2\text{O}_5 + 16\text{HI}$.

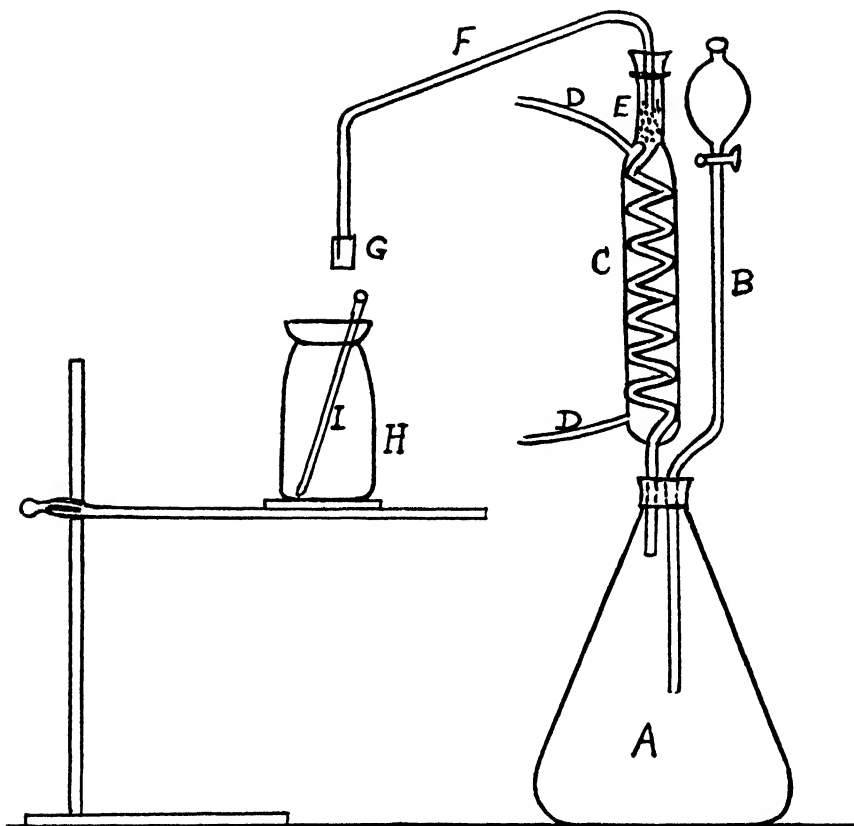
One or two pounds of fruit is washed with 3 per cent hydrochloric acid or 3 per cent sodium hydroxide, whichever may be more efficient for the removal of the spray residue. The combined washings (350–500 cc.) are placed in a 750 cc. Erlenmeyer flask (*A*). Connected with the Erlenmeyer is a worm condenser (*C*) about 6 inches long, from which the delivery tube (*F*) about $\frac{1}{2}$ cm. in diameter leads into the receiving bottle (*H*), a half-pint milk bottle being convenient. This delivery tube is joined at *G* with its lower extension (*I*) by a rubber connection. This extension tube (*I*) is for convenience, and may be used as a stirrer. It is constricted at its lower end for a length of about 2 mm. to a diameter of about 1 mm.

In the upper part of the condenser is placed the prepared cotton (*E*), impregnated with 5 per cent lead acetate and dried. Connected with the Erlenmeyer through the rubber stopper is placed a separatory funnel (*B*), used as a delivery tube for the acid and reducing reagent to be added to the solution in the flask. The attached drawing is not made accurately to scale.

The procedure is as follows:

Place the acid wash from the fruit in the Erlenmeyer. If the alkali wash is used, it is necessary to neutralize it, and add an excess of 10–20 cc. of concentrated hydrochloric acid. Place in the receiving bottle (*H*) 10 cc. of 5 per cent mercuric chloride solution made up to 100 cc. with distilled water. Add to the acidified wash from the fruit in the Erlenmeyer about 10 cc. of a reducing agent made from 2 grams of potassium iodide, 10 cc. of water, 90 cc. of concentrated hydrochloric acid and 1 cc. of the stannous chloride solution used in the Gutzeit method. In making up the reducing

ARSENIC APPARATUS.



- A—DISTILLATION FLASK, 750 CC. CAPACITY.
 H—TUBE AND SEPARATORY FUNNEL FOR ADMITTING ACID.
 C—WORM CONDENSER.
 D—TUBE FOR CONDENSING WATER.
 E—COTTON IMPREGNATED WITH LEAD ACETATE.
 F—TUBE FOR CONVEYING GAS INTO MERCURIC CHLORIDE SOLUTION.
 G—RUBBER CONNECTION FOR THE TUBE IN H.
 H—HALF-PINT MILK BOTTLE FOR MERCURIC CHLORIDE SOLUTION.

agent the potassium iodide is first dissolved in the water, after which the acid is added. Heat the solution in the Erlenmeyer to boiling. Cool to about 80°C. Connect the apparatus as indicated, loosen the stopper of the Erlenmeyer, and pour into it quickly about 25 grams of granulated 30 mesh zinc; insert the stopper at once, making sure that the whole apparatus is tight. The very poisonous nature of the arsine should be remembered. The reaction should start vigorously within a few minutes. Add further amounts of the hydrochloric acid solution of the reducing substances through the separatory funnel, adding drop by drop or faster if necessary to get a lively reaction and a fast evolution of hydrogen. No appreciable loss of arsenic through the mercuric chloride solution need be feared. The presence of the arsenic is indicated in the mercuric chloride solution by a cloudiness and soon by a precipitation. The reaction is continued for about 1 hour, or until the zinc is all used. The apparatus is disconnected at G, and the bottle with the tube is taken for titration of the arsenic by a standardized iodine solution.

Before beginning the titration, add about 10 cc. of a 15 per cent potassium iodide solution to form the double soluble potassium-mercuric iodide. Then add an excess of about 25 per cent of the iodine solution, passing the first part of the iodine through the small delivery tube in order to dissolve any arsenic that may have been deposited in this tube. The requisite excess of iodine solution is judged by the color developed by the iodine. This depth of color should be observed by running known solutions of arsenic, which also is necessary to familiarize oneself with the operations and to test the efficiency of apparatus. It is advisable to note whether all the precipitate in the receiving bottle is completely dissolved. The delivery tube may be used as a stirrer, but it may be necessary to use also a policeman to accomplish complete solution.

Run in a standard arsenic solution to slight excess, using starch as an indicator. Add about 1 gram of bicarbonate of soda, and titrate the unknown back to a blue color with a standardized iodine solution. The difference between the total volume of iodine and arsenic solutions used, multiplied by the arsenic equivalent of the iodine, gives the amount of arsenic present in the unknown solution.

COMMENTS.

In this work the referee used an arsenic solution of 0.4 gram of As_2O_3 per liter, slightly weaker than 0.01 *N* solution, and the iodine of approximately the same strength, and standardized against the arsenic solution. The arsenic (As_2O_3) equivalent of the iodine solution when titrating AsH_3 to As_2O_5 is one-fourth of that when titrating As_2O_3 to As_2O_5 . The iodine is standardized against the arsenic and the result is divided by 4 to get the arsenic equivalent in this titration from arsine to arsenic oxide. When using 700 grams of apples, each cc. of iodine consumed is equivalent to 0.001 grain of As_2O_3 per pound when the iodine solution is exactly equivalent to the above arsenic solution. Apples or other fruit with 0.01 grain of As_2O_3 per pound would take a titration of 10 cc. in the method outlined. This large volume of iodine titration is, of course, very desirable.

In the development of this method over 200 determinations were made. Many of these were made on the known amount of arsenic added to water. Metallic aluminum and magnesium were tried as a substitute for zinc, but they were discarded for reasons of efficiency or economy. As reducing agents hydrazine sulfate and potassium bromide were tried together, also hydrazine sulfate and potassium iodide. Good recoveries were made generally on pure solution. When fruit dip solutions were used, the results were not so satisfactory. In the experiments using fruit, the apples or pears were first washed with acid or alkali to remove the arsenic spray residue present. The fruit was then washed the second time, and these solutions were used for the experiment, known amounts of arsenic being added after it had been oxidized to the arsenic form by iodine, in order to simulate the condition of the arsenic in the spray residue. It was necessary, of course, to run a blank on the reagents and the apple wash used. The writer had the collaboration of F. A. Vorhes of Seattle, and of L. H. McRoberts

and L. A. Salinger of San Francisco. He also wishes to acknowledge the advice and assistance of H. J. Wichmann of this laboratory. All the results so far obtained have not been entirely satisfactory, although with water solutions containing no organic matter results are generally good. This is largely a report of progress. It is hoped to further solve the problem of the apparent interference of the organic matter when the washings are used directly for the determination. It is apparent, however, that the method may be used successfully when applied to the acid digestion of various food products.

Some of the results obtained are given in the following table:

Results on known solutions.

| ANALYST | As ₂ O ₃ ADDED mg. | As ₂ O ₃ FOUND mg. | RECOVERY per cent |
|----------|---|---|----------------------|
| Vorhes | 2.80 | 2.73 | 96.1 |
| " | 4.00 | 3.82 | 95.5 |
| " | 3.60 | 3.50 | 97.2 |
| " | 7.00 | 6.91 | 98.7 |
| " | 10.00 | 9.65 | 96.5 |
| " | 9.00 | 8.85 | 98.3 |
| " | 4.00 | 3.91 | 97.8 |
| " | 5.00 | 4.88 | 97.2 |
| " | 1.60 | 1.57 | 98.1 |
| " | 0.40 | 0.30 | 75.0 |
| " | 1.60 | 1.30 | 81.3 |
| " | 2.00 | 1.85 | 92.5 |
| Salinger | 4.00 | 3.95 | 98.7 |
| " | 4.00 | 3.79 | 94.8 |
| Taber | 4.00 | 3.886 | 97.1 |
| " | 2.00 | 1.988 | 99.4 |
| " | 2.60 | 2.36 | 90.7 |
| " | 1.00 | 0.975 | 97.5 |
| " | 0.40 | 0.423 | 105.7 |
| " | 0.40 | 0.403 | 100.8 |
| " | 0.80 | 0.746 | 93.3 |

It might be added that in some of these determinations the whole of the reducing solution was added at once and the sample was heated to boiling, a variation of the method given. Either procedure gives good results in pure solutions, but not in alkaline wash from fruit.

When using the acid or alkali wash from the fruit to which known amounts of arsenic were added, variable results were secured, due, doubtless, to the organic matter present. On 11 determinations results varying from about 88 to 102 per cent were obtained; the average was about 97 per cent. On 10 samples of alkali fruit wash McRoberts obtained recoveries varying from 86.8 per cent to 106.5 per cent, the average being 93.3 per cent. In other work Vorhes obtained a somewhat wider range of results.

It is interesting to note the results obtained on some samples of sprayed fruit in this laboratory, in which the Gutzeit results may be compared with those obtained by the method here given of mercuric chloride reduction by arsenic.

| SAMPLE | RESULTS BY GUTZEIT | RESULTS BY PRESENT METHOD |
|--------|--------------------------------------|---------------------------|
| A | { 0.014 grain per lb. 0.015 " " " | 0.016 grain per lb. |
| B | 0.014 " " " | 0.010 " " " |
| C | 0.005 " " " | 0.0086 " " " |
| D | 0.288 mg. | 0.302 mg. |
| E | { 0.016 grain per lb. 0.02 " " " | 0.02 grain per lb. |
| F | 3.5 p. p. m. | 4.8 p. p. m. |

RECOMMENDATION¹.

It is recommended that a study of the method be continued, particularly for the purpose of eliminating the variations in results due to the organic matter.

REPORT ON BORON.

By O. F. KRUMBOLTZ (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*.

A review of the present official method² for the determination of boric acid in fertilizers showed that the recoveries of pure boric acid varied from 95 to 97 per cent. It was further found that the methyl alcohol, which was recovered for use in subsequent determinations, had carried off about 5 per cent of the boric acid. This error was considerably reduced by using a large excess of alkali. Too much alkali, however, dissolved a little of the boric acid from the glass. Further study is needed on this method to standardize the best conditions for distillation.

Much time was spent this year in developing what promised to be a satisfactory quantitative separation of boric acid from other matter, based upon the distillation of boric acid in a current of superheated steam. The original experimentation along this line appears to have been done by P. Tschijewsky³.

Superheated steam was obtained by passing steam from an outside source through a coiled tube in a copper cylinder that contained vapors from a boiling kerosene fraction sufficient to heat the steam to a temperature of 180°–190°C. The kerosene in the bottom of the cylinder was heated by an electric resistance coil. Through the top of the cylinder was set a large test-tube which contained the boric acid to be distilled. The superheated steam was led into this distilling tube where it passed over the boric acid, thence out into a spray trap and condenser. The cylinder was further provided with an air-cooled condenser which prevented the accumulation of pressure.

By using this method with the above-mentioned apparatus it was

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 72 (1930).

² *Methods of Analysis*, A. O. A. C., 1925, 18.

³ *Arch. Sciences Genève* (3), 12: 146 (1884); *C. A.*, 6, 3213 (1912), 7, 2502 (1913), 20, 25, 1965 (1926).

possible to recover from 99.5 to 100.2 per cent of pure boric acid, or acidulated borax.

In the case of samples containing organic matter, it was necessary first to add alkali; then ash the substance in a muffle at a low red heat, after which it was acidified and distilled. With some of the simpler mixtures, such as dextrose and boric acid, the recoveries varied from 99.0 to 99.7 per cent by this method. In such a case as wheat flour, for example, a loss of several per cent of boric acid was noted, indicating a greater retention of the boron by the ash of the flour than was noticed in other samples. More detailed study of this method, as applied to a wider range of samples, is needed before specific recommendations can be made.

Better methods for detecting and estimating minute quantities of boron are needed, and efforts should be directed to this end during the coming year.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the present boron methods be more thoroughly studied.
- (2) That the method for distilling boron with superheated steam be studied further.
- (3) That methods for estimating minute quantities of boron be studied.

REPORT ON TIN.

By U. LIDDEL (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The tin content of foods may be ascertained by gravimetric and volumetric determination, electrolytic separation, potentiometric titration or spectrographic identification.

It is quite evident that spectral analysis is most satisfactory for all metals in foods because it is exceedingly accurate, rapid and simple and also leaves a permanent record. However, this method requires an expensive type of apparatus, the spectrograph, seldom found in any but the best equipped laboratories, and an operator who understands the theory well enough to interpret the results correctly.

Electrolytic methods present various difficulties, and they are not dependable for an accuracy within the range of other methods (16), (17), (18)². Potentiometric titration is very accurate, being a variation of the usual volumetric procedure. The other methods are presented with the tentative methods of this association³.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 72 (1930).

² See references.

³ *Methods of Analysis*, A. O. A. C.

Gravimetric determination is simply the purification and weighing of an insoluble compound of tin. Besides the official method, Misk (14) presents a method for the determination of tin as the stannic oxide and Pinkus and Claessens (15) use cupferron for the precipitation. However, gravimetry presents many possible sources of error and is not accurate beyond balance limitations.

Most of the references in the literature discuss volumetric determinations which offer the easiest, simplest, and most accurate of the common methods of analysis. It is essentially the reduction of the tin to the stannous state after the removal of interfering constituents of the sample followed by the oxidation of the stannous tin to stannic tin with a suitable oxidizing agent, standard iodine solution being quite satisfactory and the one most generally used. Potassium dichromate is frequently used and is more stable in storage. Ferric chloride is also used (2), (3), but its preparation and standardization present difficulties, and it is not so stable as other solutions. Chloramine and potassium permanganate (7) have also been used (13). Several reducing agents are satisfactory: the tentative method specifies aluminum; Kolthoff and Heidje (1) recommend reduced iron powder; and Evans (5) uses lead. Since stannous salts are readily oxidized by air, it is necessary to keep the test solution in an atmosphere of an inert gas, like carbon dioxide. The tentative method and several others specify carbon dioxide from an outside generator, but the associate referee has found no objection to the use of a piece of marble in the titration flask, which eliminates the extra apparatus for external CO₂ generation.

Volumetry requires the use of a colorimetric indicator for the end point of the oxidizing reaction (starch iodide is generally used), but potentiometric titration eliminates the use of starch and is much more sensitive. The procedure is practically the same for both except that the potential difference between an electrode in the sample being titrated and another in the untitrated portion of the sample is recorded and plotted against the amount of reagent used. A maximum of potential indicates the end point of the reaction (19), (20), (21).

For many years (9) it has been recognized that "wet-ashing" of samples is an objectionable and time-consuming procedure, and a substitution for this would be desirable. Glassman and Barsutzkaja (6) successfully used an incineration.

In several experiments the direct extraction of the tin from samples of canned pumpkin with hydrochloric acid was attempted, but enough soluble colored matter was extracted to make an iodine end point invisible.

The following objections are offered to the present tentative methods: (1) The precipitation of the tin as sulfide is a time-consuming process and introduces possible errors due to incomplete precipitation and loss

of residue in transfer to the titration flask; (2) the presence of asbestos and the Gooch bottom are undesirable features in the titration flask. The Kipp generator for carbon dioxide adds unnecessary complications to apparatus required, and it is believed that a piece of marble may give an equally satisfactory atmosphere of carbon dioxide.

The following method which has given a satisfactory determination of tin (after the tin was in solution and organic matter removed) in several experiments is suggested:

The volume of the solution containing the sample was changed to 100 cc. by evaporation or dilution, and 57 cc. of concentrated hydrochloric acid was added to make the solution four times normal in acidity. Two drops of 1 per cent SbCl_3 solution were added and then approximately 0.2 gram of reduced iron powder, and the solution was boiled until the iron was completely dissolved, the flasks being covered with funnels. A small piece of marble was next added, and the solution was cooled quickly in ice to approximately 20°C . A measured quantity of standard iodine solution was added, followed by immediate titration of the iodine excess with standard 0.2 *N* sodium thiosulfate solution.

It is regretted that no definite results can be given at this time; it was necessary to drop the experimental part of the work. However, these notes are offered with the hope that they may be of some benefit to other workers. A short list of references is attached.

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REPORT ON COPPER.

By REED WALKER (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

A review of the literature of this association pertaining to the work done on metals in foods¹ showed that no work had been reported on copper during the past fifteen years, although it has been recommended annually that methods for determining copper in foods be studied.

Because no record of work done prior to the adoption of the present tentative method² was found, it was thought wise to make a thorough review of this method to determine its fitness for retention.

In order to simulate the usual conditions, a "stock" solution was made containing all the metallic salts that may be found in common foods in about the same proportions. The present tentative method was tested thoroughly with known quantities of copper and was found to be unsatisfactory for the following reasons:

1. The wet digestion used in destroying the organic material is trustworthy but very tedious.
2. The precipitation of the copper from solution with hydrogen sulfide is also tiresome and of doubtful value for such small quantities of copper.
3. The digestion of the copper sulfide precipitate and filter paper with nitric and sulfuric acid leads to high results³.

Many modifications of the tentative method were made this past year in an attempt to overcome the above-mentioned difficulties. As a substitute for the wet digestion, ashing in an electric muffle was tried; it proved to be satisfactory as far as noted. Elvehjem and Lindow⁴ recommend this method of destroying organic material, as do King and Etzel⁵.

Instead of precipitating the copper from solution with hydrogen sulfide, a saturated solution of sodium thiosulfate was used. This gave a coarse granular precipitate which was easy to handle and did not need to be washed with hydrogen sulfide water. The time of precipitation was materially reduced.

In place of digesting the copper sulfide precipitate in nitric and sulfuric acids it was ignited, and the residue was taken up in nitric acid. This did away with the objectionable acid-fuming and gave a satisfactory solution to titrate.

The procedure that proved most satisfactory was as follows:

Neutralize the solution, obtained either by the wet digestion method or by dissolving the ashed sample in hydrochloric acid, with ammonia. Add 5 cc. of concentrated sulfuric acid, dilute the solution to 200 cc., and boil it for 1 minute. Cautiously add 10 cc. of a saturated solution of sodium thiosulfate. Continue the boiling until the

¹ *This Journal*, 1915-29.

² *Methods of Analysis*, A. O. A. C., 1925, 175.

³ S. Popoff, M. Jones, C. Tucker, W. W. Becker, *J. Am. Chem. Soc.*, 51, 1301 (1929).

⁴ *J. Biol. Chem.*, 81, 438 (1929).

⁵ *Ind. Eng. Chem.*, 19, 1004 (1927).

precipitate coagulates and the liquid becomes practically clear. (A few cc. of 10 per cent ammonium sulfate solution may be added to hasten the coagulation.)

Filter the precipitate and wash it six times with hot water. Fold the precipitate within the filter paper, place in a small crucible, and ignite in an electric muffle at dull red heat. Dissolve the residue in 1 cc. of nitric acid (sp. gr. 1.12) and transfer into a 100 cc. Erlenmeyer flask. Take the solution to dryness on a steam bath. Dissolve the copper nitrate in 20 cc. of water and add an excess of ammonium hydroxide. Boil the solution until it turns light brown, then add a slight excess of glacial acetic acid. Boil the solution for 1 minute, cool it to room temperature, and add 2 grams of potassium iodide dissolved in enough water to make the final solution 50 cc., including the standard thiosulfate solution used in titrating. Titrate the free iodine with 0.01 *N* sodium thiosulfate solution until the end point is nearly reached, then add 2 cc. of freshly prepared 1 per cent starch solution. Continue the titration drop by drop to the end point.

It is recommended¹ that the method proposed for the determination of copper in foods be studied collaboratively.

REPORT ON FRUIT PRODUCTS.

By H. J. WICHMANN (U. S. Food and Drug Administration, San Francisco, Calif.), *Referee*.

The work on fruit products this year was planned as a continuation of that of last year. However, the resignation of Doris H. Tilden as Associate Referee on Ash in Fruit Products caused a realignment. V. B. Bonney was appointed to the vacancy. Official duties away from headquarters prevented Bonney from conducting the collaborative work on the analysis of fruit ashes that had been planned. Even though there is no report on fruit ash by the associate referee, some ash work was done by the members of the referee's laboratory. Mrs. Tilden had made up an ash solution of known composition, and members of the San Francisco Station are still using it in a double-barreled investigation designed to test both the methods and the analysts. The methods reported last year have been altered somewhat in an effort to make them more exact. Precipitations are conducted at approximate pH values determined by color changes with brom cresol green. The results in the main have been satisfactory, and it is believed that the associate referee will be able to send out a better method with collaborative samples next year. Nothing was done on the subject of the determination of chlorine in fruit products.

Reports of former referees have indicated that the results on solids by drying are too high by 5 per cent of the sucrose inverted during the drying and that the amount of inversion is not unappreciable. Referee Gowen determined that when the sucrose and acid solution was dried with asbestos or sand as absorbent, the inversion was less and hence the

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 72 (1930).

error was also less. He corroborated the observation of the referee that the refractive method gave the truest solids percentages in solutions of sucrose and organic acid, but indicated this might not be true in the case of sweetened fruit juices or other fruit products when substances besides sugar and acid were present. It seemed to the referee that before the question of what is the best method for determining solids in such substances as sweetened fruit products could be solved, it would be necessary to reduce the inversion during drying to a minimum, and he was curious to know why the inversion was less when an absorbent was used. Did the material of the dish have any catalytic effect on the inversion, or did the absorbent merely expose less of the sugar to this action? Kathryn Breen of the New York State Department of Agriculture made some experiments, using dishes of different materials with and without absorbents. She corroborated the work of Gowen to the effect that an asbestos or sand absorbent caused less inversion in the drying of a solution of sucrose and tartaric acid, and, therefore, a closer agreement with the actual percentage of solids and solids determined by refractive methods. She found this to be true regardless of the material of the drying dishes. Apparently, then, the smaller amount of inversion when an absorbent is used is due to the greater separation of the molecules of sugar and acid and not to the catalytic action of the material of the dish. Since tartaric acid is a fairly strong organic acid, Miss Breen suggested that citric acid should cause less inversion. Since citric acid crystallizes with one molecule of water of crystallization, it was necessary to determine what effect vacuum drying at 70°C. would have on its composition. Miss Breen reports that citric acid loses all its water of crystallization in the vacuum at 70°C. Therefore the way is open to determine the relative inverting action of tartaric acid, a relatively strong organic acid, and citric acid, a weaker acid, with and without the presence of an absorbent. The referee believes this relative action should be studied on solutions of sucrose containing 0.1 mol. of the acids and perhaps also with different amounts of acids, but with the same hydrogen-ion concentration. After more information has been obtained on the mechanism of the inverting action of the acid on the sugar in the drying process, the referee is of the opinion that the inversion may be reduced to a minimum and a check be obtained on the percentage of solids of such products as sweetened fruit juices and jellies by two independent methods, such as solids by drying and by refractive methods. It could then be determined which method is the more accurate and dependable under varying conditions.

B. G. Hartmann, the Associate Referee on Fruit Acids, reports that he¹ has modified his method for citric acid¹ and finds that it is not neces-

¹ *This Journal*, 11, 257 (1928).

sary to adjust the solids and citric acid content. He informed the referee that acceptable methods for the determination of citric and tartaric acid by (1) the Kling method and (2) a modification of the potassium acid tartrate method and total acids by a lead acetate method have been perfected and are ready for publication¹. The study of methods for the determination of malic acid has not been completed, although the results are promising. The referee does not know the details of these methods and makes no definite recommendations except to say that they should be tested at the earliest possible time and earnestly requests that the next associate referee make particular effort to secure wide participation in the collaborative study.

Last June Philip B. Myers and George L. Baker published Bull. No. 160, University of Delaware Agricultural Experiment Station, entitled "The Rôle of Pectin—The Extraction of Pectin from Pectic Materials". The referee wishes to quote parts of the summary that have a bearing on the tentative pectin methods.

The jelly grade of a pectin reaches an optimum value when the pectin is extracted at a hydrogen-ion concentration of approximately 2.40. This optimum point is independent of the titratable acidity of the extracting medium and of the nature of the acid used. Extractions at hydrogen-ion concentrations greater than that represented by a pH of 2.40 results in a sharp decline in the jellying power of the pectin, indicating a hydrolytic action on the pectin.

The jelly grade of a pectin decreases as the time of boiling during extraction increases. Boiling from 5–15 minutes results in a sharp decline in jelly grade; boiling from 15–30 minutes has scarcely any effect upon the jelly grade of the resulting pectin; boiling from 30–60 minutes sharply decreases the jelly grade and boiling from 60–180 minutes the decrease in jelly grade is more gradual and is a straight line function of the time of boiling.

The yield of pectin depends upon the hydrogen-ion concentration at which the pectin is extracted rather than upon the titratable acidity. Increasing the hydrogen-ion concentration beyond a pH of 4.0 results in a sharp increase in yield of pectin. With tartaric acid the maximum yield is obtained at pH of 2.0; with hydrochloric acid the optimum point occurs approximately at a pH of 1.45. Increasing the hydrogen-ion concentration beyond these points results in a sharp decrease in the yield of pectin, indicating that the pectin is hydrolyzed and that some of the hydrolytic products are insoluble.

The yield of pectin also depends upon the time of boiling during extraction. Boiling from 5–60 minutes, the yield increases rapidly; boiling from 60–120 minutes, the increase is not so pronounced; while boiling longer than 120 minutes results in practically no increase in yield above that obtained by boiling 120 minutes.

Regardless of the amount and nature of the acid used, a pectin of maximum jelly units is obtained when it is extracted at a hydrogen-ion concentration of pH 2.15. When the hydrogen-ion concentration is greater or less than that represented by a pH of 2.15 there is a sharp decline in jelly units.

A pectin of maximum jelly units is obtained when the extraction is accomplished by boiling 30 minutes. A boiling period shorter or longer than 30 minutes results in a decrease in jelly units.

¹ *This Journal*, 13, 99, 103 (1930).

The A. O. A. C. method depends upon the natural acid present in the fruit or fruit product and on heat to extract all the pectin. Only in the case of citrus peels and of certain figs has it been found advisable to add extra acid for the purpose of extracting a maximum quantity of pectin as measured by the alcohol precipitate and pectic acid. Naturally, then, pectin has been extracted at various hydrogen-ion concentrations. It is of interest to note that Myers and Baker found that boiling the pectic material for 60 minutes extracted the greater part of the pectin, although such time of boiling lowered the jelly strength of the extracted pectin. This corresponds with the experience of the referee in so far as alcohol precipitate and pectic acid values are concerned. Alcohol precipitate and pectic acid measure the quantity of the pectin, but not the quality. In this respect these determinations are similar to nitrogen determinations in the case of flour, the value of which for certain purposes being determined by other factors. But if the yield of pectin also depends upon the pH of the extracting medium, a very interesting issue is raised in connection with the A. O. A. C. methods, because undoubtedly the pH of these extractions has not always been the optimum one. In fact, a pH of 2 seems surprisingly acid. Myers and Baker recommend that for the purpose of extracting the greatest number of jelly units (yield \times jelly strength) in one extraction, the pH be adjusted to 2.15 and the boiling continued for 30 minutes. This proposal, if adopted by the A. O. A. C., would cut the time in half and probably increase the pH of extraction in the case of many fruits. The idea of a definite pH for extraction is of intriguing interest, and if followed might bring important developments in pectin determinations and fruit analysis. The referee is of the opinion that the effect of a definite extraction pH and curtailment of time of boiling on alcohol precipitate, pectic acid, and ash percentages should be studied for a variety of fruits, not only in citrus albedos. Adjustment of pH in the case of many fruits would necessarily have to be by electrometric methods owing to the depths of natural color.

Another point that should be kept in mind in any future study is the possible effect of sugar on the extraction and precipitation of pectin. Acids, heat and time of boiling undoubtedly have an effect on the strength of pectin, but the referee is of the opinion that such action is considerably reduced in the presence of sugar. Sugar is found in all fruits and in some fruit products in a considerable amount. Myers and Baker worked with lemon albedo whose carbohydrate content was perhaps unknown both as to quantity and kind. Therefore some information is needed on whether the recommended procedure in the case of lemon albedo produces optimum yields and jelly units in the case of other fruits and fruit products containing varying percentages of sugars.

If extractions of pectin from fruits were always made at a definite pH, the ash and composition of the ash from a given fruit might be more

uniform than it is at present. It is at least an interesting speculation and might bear some investigation.

RECOMMENDATIONS¹.

1. The referee recommends that the work on the determination of solids in solutions of sucrose and organic acids, as for example in sweetened fruit juices and other fruit products, be continued by a new associate referee.

2. He recommends that the investigation of methods for the determination of the major bases as well as chlorine in plant or fruit ashes be continued.

3. Particular stress is recommended on the collaborative testing of the fruit acid methods.

4. It is also recommended that a new associate referee be appointed to begin a study of the effects of a definite pH of extraction of the pectin on the alcohol precipitate, pectic acid, and ash of fruits and fruit products.

REPORT ON FRUIT ACIDS.

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*.

In the course of work devoted to the development of methods for the determination of malic acid, in the absence of a specific precipitant for the acid, it was necessary to test the isolated malic acid for the acids commonly occurring in fruits and fruit products. The first step, therefore, was to devise adequate methods for citric and tartaric acids, which would permit the determination of the small quantities of these acids present as contaminations in the isolated malic acid.

Three papers have been prepared for publication in *The Journal* of the association: (1) The Use of Lead Acetate in the Determination of the Acidity of Fruit Products; (2) The Determination of Citric Acid in Fruits and Fruit Products, and (3) The Determination of Tartaric Acid in Fruits and Fruit Products. It is expected that these papers will be published² within a short time.

If the experiences of F. Hillig and the associate referee are considered, it is believed that the methods should be investigated collaboratively next year, and it is so recommended³.

No report on ash in fruits and fruit products was given by the associate referee. See report of the Referee on Fruit Products.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 72 (1930).

² *This Journal*, 13, 99, 103 (1930).

³ For report of Subcommittee C and action of the association, see *This Journal*, 13, 72 (1930).

No report on canned foods was given by the referee.

The address of the president, H. B. McDonnell, was published on p. 19 of Vol. 13 of *This Journal*.

E. M. Bailey.—I am sure we are indebted to Dr. McDonnell for his presentation of the detailed study of this very interesting subject. Of course, we very much regret that Dr. Wiley, by reason of his ill health, will not be able to give us his usual address. However, Dr. B. B. Ross¹ has a message to deliver to us concerning him.

B. B. Ross.—Mr. Chairman: The committee consisting of Messrs. Lythgoe, Brackett and myself, called at the residence of Dr. Harvey W. Wiley, our beloved honorary president, yesterday afternoon to express our solicitude and interest in the progress of his case and at the same time express our extreme and sincere regret that he was unable to be present at this meeting of the association, as had been his custom for forty-five years in succession. He has never failed to attend a single meeting. Mrs. Wiley was extremely appreciative of the call of the committee and of our interest in the Doctor's illness. We found that his condition is very serious, indeed, and we were not able, and in fact did not expect, to see the distinguished sufferer himself. We could only express our interest and solicitude and our best wishes for his recovery, though I might say that his illness is extremely grave.

In this connection I thought it not amiss, after mentioning the matter to several members, to make it known that my own institution, the Alabama Polytechnic Institute, at Auburn, Alabama, has recently seen fit to honor Dr. Wiley in connection with the construction of our new chemistry building. This building is trimmed with Bedford stone and the architect provided that in the stone at various angles a place should be made for the inscription of the names of eminent chemists, both from the old world and this side of the water. On two prominent corners there have been inscribed the names of Wiley and Johnson, by reason of the fact that they are conspicuous representatives of the eminent group of chemists whose lives have been notable on account of their achievement in behalf of human welfare. I had a little folder made showing a photograph of the name of Wiley overlooking the park in the direction of the State laboratory, and I brought this on, not knowing of Dr. Wiley's illness, and left it at his house yesterday. My institution is extremely glad to have a part in honoring these two men who are conspicuous exemplars of chemistry in the service of humanity.

Dr. Wiley died on June 30th, aged 86 years. An obituary will be published in a later issue of *This Journal*.

¹ Died in April, 1930.

SECOND DAY. TUESDAY—AFTERNOON SESSION.

REPORT ON CEREAL PRODUCTS.

By J. A. LECLERC (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

Last year's report¹ of the general referee contained an historic résumé of the activities of this association as they related to cereal products. It included the names of the referees and their terms of office, and also a list of the various methods which had been adopted officially or tentatively since 1901, when cereal products were for the first time considered by this body.

As the result of last year's work (1928-9), the association added the following methods of analysis as official first action: In flour, (1) sampling, and (2) water-soluble nitrogen precipitable by 40 per cent alcohol; in baked products, (1) total solids in an entire loaf, (2) chlorides in ash, (3) moisture, (4) crude fiber, (5) organic and ammoniacal nitrogen; in alimentary pastes, (1) moisture, (2) water-soluble protein precipitable by 40 per cent alcohol, and (3) crude fiber. The following methods were adopted as tentative: in flour, the F. A. C. method for the determination of unsaponifiable matter in fats, (2) glutenin; in baked products, (1) fat by acid hydrolysis and (2) standard experimental baking test.

Too much credit cannot be bestowed upon the associate referees and their collaborators for the progress thus made in this field of work.

During the past year more attention has been paid to baking and baked products than formerly. A new line of work has likewise been inaugurated, viz., the study of certain methods of analysis used by foreign governments in testing flour imported from this country.

The work of the past year may be summarized as follows:

SAMPLING OF FLOUR.

Associate Referee H. Runkel has recommended that the method for sampling be studied with respect to each determination (nitrogen, ash, etc.), and that the word "steel" now applicable to the "trier" used in sampling flour be changed to "metal".

GASOLINE COLOR VALUE AND ASH.

D. A. Coleman, associate referee, reports, as the result of a questionnaire distributed among various cereal chemists, that since the method

¹ *This Journal*, 12, 378 (1929).

for gasoline color value is used very little it should not be subjected to further refinement. He also studied the use of salts of the rare earths as a means of hastening the ashing process, and finds that this method is not yet satisfactory for all classes of flour. Further work along this line is recommended.

GLUTENIN IN FLOUR.

Associate Referee M. J. Blish conducted special research on the nature of glutenin, the results of which seem to indicate that so-called "glutenin" is not a distinct chemical entity. The associate referee therefore recommends that collaborative work involving methods of analysis as heretofore conducted be discontinued.

HYDROGEN-ION CONCENTRATION.

Associate Referee C. H. Bailey submitted a sample of flour to each of nine collaborators for the determination of hydrogen ion by two methods: (1) hydrogen electrode, (2) quinhydrone. He recommends that the phraseology of the present method be amended so as to permit the use of either method for the measurement of the pH of flour extracts.

DIASTATIC VALUE OF FLOUR.

Associate Referee A. H. Johnson studied the effect on the diastatic value of the difference in size of particles of flours before and after extraction with ether and the H-ion concentration of flours of different grades. He recommended that further study be made on the influence of the H-ion concentration.

STARCH IN FLOUR.

Associate Referee L. H. Bailey conducted collaborative studies of (1) the Rask and (2) the Hartmann and Hillig methods. The results indicate that further studies of these methods should be made.

FLOUR-BLEACHING CHEMICALS.

G. C. Spencer, associate referee, obtained results on the determination of chlorine in bleached flour by Seidenberg's method from eight collaborators and recommends that this method be adopted as tentative.

FOREIGN METHODS OF ANALYSIS.

Associate Referee C. H. Bailey made a thorough study of the literature, European and American, relating to the determination of acidity in flour, and as a result he recommends that collaborative study of this method be carried on so as to compare the present tentative method with that of alcohol extraction, which is generally used in Europe.

SAMPLING OF BREAD AND DETERMINATION OF MOISTURE.

L. H. Bailey, associate referee, followed the recommendations of the committee by making a study of this problem with breads of different types. Four distinct commercial breads were sampled by cutting each one lengthwise and again crosswise and determining the moisture in each of the four sections. The results of this work showed marked concordance between the figures for moisture in the whole loaf and figures for moisture in each half-loaf. The results indicate that one-half of the loaf is a sufficiently large sample to determine the moisture content of a loaf of bread, and it is recommended that this subject be taken up collaboratively.

LIPIDS AND FAT IN BAKED PRODUCTS.

Associate Referee Samuel Alfend conducted no collaborative work on this subject this year, but as a result of work done in his own laboratory he recommends (1) that further collaborative work be carried on with the methods for the determination of fat in baked products, and (2) that further work be likewise carried on for the determination of lipoids in bread.

CRUDE FIBER IN ALIMENTARY PASTES AND BAKED PRODUCTS.

Associate Referee Sterling tested the applicability of the official method (for flour) to baked products and alimentary paste and has found that the technic of the method as used for flour is entirely satisfactory for baked products and alimentary pastes. He recommends (1) that the official method for the determination of crude fiber in flour be made official for air-dried baked cereal products (first action), and (2) that the official method for the determination of crude fiber in flour be made official (first action) for alimentary pastes.

FAT BY ACID HYDROLYSIS IN ALIMENTARY PASTE.

Associate Referee Alfend reports that this official (first action) method has been checked by collaborative study and used without objection during the past two years in his own laboratory and elsewhere, and as a result recommends its adoption as official (final action).

LIPIDS AND LIPOID PHOSPHORIC ACID IN ALIMENTARY PASTE.

This tentative method (official first action) has also been checked and used satisfactorily by Associate Referee Alfend and others for the past two years. The associate referee recommends, therefore, that it be adopted as official (final action).

No reports on milk solids in milk bread, rye flour in rye bread, and organic and ammoniacal nitrogen in baked products were made.

EXPERIMENTAL BAKING TESTS.

Associate Referee Blish conducted extensive collaborative work, and as a result he feels that definite and substantial progress has been made on this subject, which is one involving extensive difficulties and complications. He recommends that the association continue to collaborate along the present lines with the A. A. C. C. toward the establishment of a standard and official experimental baking test.

WATER-SOLUBLE PROTEIN PRECIPITABLE BY 40 PER CENT ALCOHOL.

Associate Referee Alfend reports that the results of collaborative work heretofore obtained has indicated the need for the thorough study of this method. After considerable work, he concluded that the method adopted is not always reliable and that it needs revision. This method has already been adopted as official (final action) in the case of flour and as official (first action) in the case of alimentary paste. His recommendation is that this method be further studied both with flour and with alimentary paste.

UNSAPONIFIABLE MATTER IN THE FAT OF FLOUR, ALIMENTARY PASTE, AND BAKED PRODUCTS.

Associate Referee Alfend reports that the method for the determination of unsaponifiable matter in fat of flour, alimentary paste, and baked products has failed during the three years' collaborative work to give uniform results and recommends that no further collaborative work be attempted on this subject until a thorough study has been made to determine the factors that are the cause of these unsatisfactory results.

There was no report on collecting and preparing samples of alimentary paste for analysis, nor on moisture in alimentary paste.

RECOMMENDATIONS¹.

FLOUR.

It is recommended—

(1) That in the tentative (official first action) method for sampling flour the word "steel" in the second line of the second paragraph be deleted and the word "metal" inserted instead, and that the method be further studied collaboratively.

(2) That the associate referee continue the study of rapid methods of ashing flour, baked products, and alimentary paste, particularly regarding the effect of the rare earths as aids thereto, the influence of temperature as well as the applicability of glycerol-alcohol mixture, etc.

(3) That the associate referee study the nature of the losses occurring when ash is fused.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 73 (1930).

(4) That special studies of the method for the determination of unsaponifiable matter in fat of flour be made by the associate referee before it is subjected to collaborative work.

(5) That special studies be undertaken regarding the method of determining glutenin in flour, pending which all collaborative work be suspended.

(6) That for the determination of the H-ion concentration, either the H electrode or the quinhydrone electrode be used.

(7) That methods for the determination of the diastatic value of flour be studied.

(8) That the Seidenberg method for the determination of chlorine in chlorine-bleached flour be adopted as tentative, and that attention be directed to the study of methods for the detection of bleaching of flour by benzoyl and other peroxides.

(9) That further collaborative study be made of the tentative method (Rask) for the determination of starch in flour, bread and alimentary paste by comparing it with the diastase method, as modified by Hartmann and Hillig.

(10) That the proposal to substitute the factor 5.83 for the factor 5.7 for converting nitrogen of wheat into protein be laid on the table.

(11) That the tentative (official first action) method for water-soluble protein nitrogen precipitable by 40 per cent alcohol in flour be further investigated.

(12) That the methods of analysis used by foreign government chemists for the testing of flour imported from this country be further studied, and that the tentative method for the determination of acidity in flour be compared with foreign methods wherein alcohol is used as the extractive medium.

(13) That no further collaborative work be done with the present method for determining gasoline color value of flour.

BAKED PRODUCTS.

It is recommended—

(1) That collaborative study be made of the tentative method for the sampling of bread to determine the possibility of utilizing only one-half of the loaf instead of the whole loaf and that different types of bread be used.

(2) That the tentative method (official first action) for the determination of total solids in an entire loaf of bread be studied collaboratively and include studies to determine the possibility of estimating the total solids in bread by utilizing only one-half of the loaf and that different types of bread be used for the experiment.

(3) That the tentative method for the determination of fat in bread by acid hydrolysis be subjected to further study.

(4) That further study be made of the methods of determining lipoids in baked products.

(5) That special studies be made by the associate referee of the tentative method (now applicable for flour) for the determination of unsaponifiable matter in the fat of bread and other baked products.

(6) That consideration be given to the development of methods for the determination of milk solids in bread, and of rye in rye bread.

(7) That the tentative method (official first action) for the determination of chlorides in baked products be studied collaboratively.

(8) That the tentative method (official first action) for moisture determination in baked products be studied collaboratively.

(9) That the tentative method (official first action) for the determination of crude fiber in baked products be studied collaboratively.

(10) That the tentative method (official first action) for the determination of organic and ammoniacal nitrogen in baked products be studied collaboratively.

(11) That further study (supplemented by collaborative work) be carried on with the tentative method of making a standard experimental baking test.

(12) That the associate referee make a record next year on the subject of total solids in an entire loaf of bread by the 130°C. air oven and other rapid methods.

(13) That consideration be given to the study of baked products other than bread.

ALIMENTARY PASTES.

(1) That the tentative method for collecting and preparing a sample of alimentary paste for analysis be further studied with the view to making it official.

(2) That the tentative method (official first action) for the determination of moisture in alimentary paste be further studied.

(3) That further study be conducted with the tentative F. A. C. method for the determination of the unsaponifiable matter in the fat of alimentary paste before it is submitted for collaborative work.

(4) That the tentative method (official first action) for water-soluble protein nitrogen precipitable by 40 per cent alcohol be further investigated.

(5) That the official method for the determination of crude fiber in flour be studied collaboratively with alimentary paste.

(6) That the tentative method (official first action) for determining total solids in alimentary paste be studied collaboratively.

(7) That the tentative method (official first action) for the determination of fat in alimentary paste by acid hydrolysis be studied collaboratively.

(8) That the tentative method (official first action) for the determination of lipoids and lipoid phosphoric acid in alimentary paste be studied collaboratively.

REPORT ON SAMPLING OF FLOUR.

By H. RUNKEL (U. S. Food and Drug Administration, Chicago, Ill.),
Associate Referee.

The association, both last year and the previous year, recommended further work before adoption of the method for sampling flour as official. Both years were spent by the associate referee in reviewing literature, in personal discussions and in correspondence to discover the most doubtful phase of the method as written. However, the accuracy of the method as applied to moisture did not seem to be questioned, and except for the policy in *Methods of Analysis* to give directions for sampling at the head of the chapter, thereby making the method apply to all determinations mentioned, recommendation would have been made that the method be changed to apply to moisture only. No data have been presented to show the accuracy for ash, nitrogen, and other determinations mentioned in the method book, nor have any been presented to show the accuracy for ground glass, water damage, weevil infestation, or other tests not mentioned in the book. It is doubtful if collaborative demonstration of the accuracy for all purposes is desirable or possible. However, limitation of the scope of the method may bring such demonstrations within the realm of possibility.

It is recommended¹, therefore, that the method "direction for sampling" be studied collaboratively with respect to other determinations now adopted by the association as official.

Since all collaborative work was done with a brass trier, and the principal essential appears to be that the trier be smooth, it is recommended that the word "steel" be deleted and the word "metal" be substituted in the second line of the second paragraph.

REPORT ON GASOLINE COLOR VALUE AND ASH DETERMINATIONS IN FLOUR.

By D. A. COLEMAN (Milling, Baking and Chemical Laboratory, Grain Division, U. S. Bureau of Agricultural Economics, Washington, D. C.), *Associate Referee.*

Before proceeding with collaborative work relative to the gasoline color value of flour, a survey was made to determine whether the method was of sufficient importance to justify extensive study. A questionnaire was sent to several prominent cereal chemists in the important milling centers in order to determine who was using the test, how extensively it was used, and if there was any prospect for its increasing popularity.

The replies to the questionnaire were almost identical. The test is

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 73 (1930).

rarely used in any practical way by any of the large mills. In a crude way it is sometimes used by those interested in the sale of flour-bleaching equipment to demonstrate the efficiency of their products, and in a limited way by certain plant breeders to test approximately the carotin content of wheat and flour.

Moreover, as the result of the researches of Schertz¹, Ferrari and Bailey², and others, it would appear that newer and more precise methods for determining color can be devised. This fact, coupled with its limited use, would make it appear that the gasoline color value determination should not be subjected to detailed and refined modifications.

During the past year F. W. Walters, of the School of Hygiene of Johns Hopkins University, recommended the use of certain rare earth salts as a means of hastening the ashing of flour. It was demonstrated that the acetates or nitrates of cerium, lanthanum, thorium, and yttrium exhibited marked ability to hasten the combustion process. Of the many rare earth salts studied, lanthanum nitrate appeared to be the best suited to the ashing of flour, and for its use the following procedure was suggested:

Prepare two solutions of $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ in 40 per cent alcohol, the one supplying 0.5 mg. of La_2O_3 per cc. and the other 1 mg. per cc. Use the first in ashing soft wheat flours and the second in ashing hard wheat flours, as follows:

Into an ignited, tared, flat-bottomed silica dish, 65 mm. in diameter or larger, weigh 5 grams of flour. Spread this evenly over the bottom of the dish and add 10 cc. of the appropriate solution. Mix uniformly, breaking up all the small lumps by means of a light weight stirring rod. Wipe off the stirring rod with a small piece of ashless filter paper, add this to the sample, and place directly in a muffle furnace heated to bright cherry red. Leave in the furnace 30 minutes, counting the time from the moment all volatile matter is driven off. Bring the ash to room temperature in a desiccator and weigh, correcting the result by subtracting the weight of La_2O_3 used.

Note I. Small balls or lumps of carbonaceous matter remaining after 30 minutes' ashing are due to flour which did not come into contact with the lanthanum salt in mixing. While these lumps are, of course, to be avoided, they do demonstrate the effect of the lanthanum salt or rather the effect of the absence of the lanthanum salt.

Note II. Adding the solution to the charred mass instead of to the flour does not work so well, the oxide forming a coating which protects the carbon.

Before progress could be made, however, it became necessary to know what the furnace temperature was at bright red heat. As far as could be ascertained this was approximately 850°C. With this as a starting point, four straight grade flours, milled from hard red spring, hard red winter, soft red winter, and white wheat, respectively, were ashed by the method described, with the results shown in Table 1. As a standard of comparison the A. O. A. C. method was used, and also this method modified by the use of glycerol alcohol.

¹ *J. Agr. Research*, 30, 253 (1925).

² *Cereal Chem.*, 6, 457 (1929).

TABLE 1.

| METHOD | CLASS OF FLOUR | | | |
|---------------------------------------|----------------------|----------------------|----------------------|-------------------|
| | H. R. S. per cent | H. R. W. per cent | S. R. W. per cent | WHITE per cent |
| A. O. A. C., 18 hrs. at 550°C..... | 0.49 | 0.48 | 0.40 | 0.47 |
| Glycerol alcohol, 550°C., 18 hrs..... | 0.49 | 0.50 | 0.40 | 0.48 |
| Walters method, 30 m. at 850°C..... | 0.50 | 0.51 | 0.42 | 0.42 |
| “ “ “ “ 800°C..... | 0.49 | 0.49 | 0.44 | 0.42 |
| “ “ “ “ 750°C..... | 0.50 | 0.49 | 0.43 | 0.43 |

It would appear from the data presented in Table 1 that as described the method is not applicable to all classes of flour. With the hard wheat flours at a temperature from 800°C. to 850°C. fairly close comparisons were obtained by use of the Walters method and the A. O. A. C. method. Not so, however, when the soft wheats were ashed. With the soft red winter wheat flour, difficulty in ashing was experienced at all temperatures. It is also apparent that all the temperatures used were too high for ashing the white wheat flour.

Believing that smaller amounts of flour would perhaps give more satisfactory results, the experiments were repeated, 3 grams of flour and 6 cc. of alcoholic solution being used. The results of these tests will be found in Table 2.

TABLE 2.

| METHOD | CLASS OF FLOUR | | | |
|--------------------------------------|----------------------|----------------------|----------------------|-------------------|
| | H. R. S. per cent | H. R. W. per cent | S. R. W. per cent | WHITE per cent |
| A. O. A. C., 18 hrs. at 550°C..... | 0.49 | 0.48 | 0.40 | 0.47 |
| Walters method*, 30 m. at 850°C..... | 0.50 | 0.50 | 0.39 | 0.40 |
| “ “ “ “ 800°C..... | 0.48 | 0.48 | 0.41 | 0.42 |
| “ “ “ “ 750°C..... | 0.52 | 0.52 | 0.46 | 0.47 |

* 3 grams of flour—6 cc. of alcoholic solution.

On the whole, the results are somewhat better than those obtained with the 5 gram sample of flour, but again the apparent necessity for different ashing temperatures for certain classes of flour is emphasized. Whereas, the hard red spring, hard red winter, and soft red winter flour ash in fairly good form at 800°C.–850°C., comparable results in ashing the white wheat flour were obtained at temperatures not in excess of 750°C.

As the work progressed it was noticed that the ashing crucibles were changing weight after each test, and in a manner that might seriously affect the results. Whether this was due to the high temperature of ashing or to a combustion of the elements of which the crucible was made with the lanthanum oxide or the oxides in the flour ash was not determined. Pressure of other work necessitated the postponement of further studies.

The method appears to have merit, and additional studies should be made by increasing the number of flour samples and varying the technic somewhat in order to make possible the satisfactory ashing of all grades and classes of wheat flour.

RECOMMENDATIONS¹.

It is recommended that no further collaborative work be done with the present method for determining gasoline color value of flour, first, because it is not in extensive use, and second, because recent advances in the study of color in flour would make it appear that it hardly merits more refinement as other methods which measure the color of flour more precisely are on the way.

It is recommended that the study be continued with the use of rare earth salts as aids in the rapid determination of ash in flour. Such factors as temperature of ashing, size of sample, and class and grade of flour should receive more extended consideration before the merits of these materials as a means of hastening the ashing of wheat flour are decided.

REPORT ON GLUTENIN IN FLOUR.

By M. J. BLISH (Agricultural Experiment Station, Lincoln, Neb.),
Associate Referee.

Subsequent to the report and recommendations of last year there were completed in the laboratory of the associate referee some investigations on the nature of glutenin in flour that appear to necessitate a substantial revision of the present generally accepted ideas regarding the identity and individuality of this protein. Due to the situation that will be herein briefly described, the associate referee has, for the time being at any rate, deemed it advisable to discontinue collaborative work involving methods discussed in previous reports.

Recent investigations in this laboratory, detailed results of which are being prepared for publication², clearly indicate that the substance ordinarily designated as "glutenin" in flour is not a distinct and well-defined chemical individual. As customarily prepared from wheat flour or from gluten by methods involving extraction with, and exposure to, dilute alkali, according to the specifications of Osborne³, glutenin has been found to vary substantially, both in amount and in chemical constitution, according to the strength of the alkali used in its extraction and in other stages of preparation, and according to the duration⁴ of exposure thereto. Variations in chemical constitution are especially

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 73 (1930).

² *Cereal Chem.*, 6, 494 (1929); *J. Biol. Chem.*, 85, 195 (1929).

³ *The Proteins of the Wheat Kernel*. Carnegie Institution, 1907.

noticeable in the amide and basic nitrogen units as estimated by Van Slyke's¹ procedure.

Preparations of "glutenin" have been made recently by a method whereby exposure to an alkaline medium is eliminated throughout the entire procedure. These preparations are seemingly of a definite and uniform composition, as evidenced by preliminary observations. The new protein differs from glutenin as ordinarily prepared, both in physical properties and in chemical constitution. It is more colloidal in physical character, and its ultimate particles or aggregates appear to be larger and more complex than those of glutenin as prepared by the conventional methods.

Regardless of the true nature or importance of the new "glutenin", the experiments clearly indicate an irreversible alteration in the chemical structure and properties of a considerable portion of flour or gluten protein when the latter is dispersed in an alkaline medium, regardless of alkali concentration. Glutenin, as customarily prepared by alkaline extraction, is manifestly a product of the irreversible action of the alkali on a more complex protein mass, in addition to being variable in composition. There is evidence that the new preparations have undergone far less alteration during preparation than is the case where customary methods of alkaline extraction are employed.

No complete information is yet available as to the following questions:

1. Is the recently prepared protein a definite and distinct chemical entity?

2. Does it vary among different flours, either in amount, or in composition, or both, and if so, do such variations have any bearing upon differences among the bread-making characteristics of different flours?

3. Is flour protein or gluten, after all, actually composed of two or more separate, distinct and individual proteins (according to the present well-established belief), or is it not possible and likely that there is but one original mass of protein material, and that the nitrogenous substances that are obtained by the use of specific solvents and treatments represent merely "fractions" whose composition and properties, respectively, depend almost exclusively upon the methods used in their preparation?

There is occasion for much further investigation that will doubtless eventually lead to a substantial revision of present-day ideas regarding the nature of wheat flour protein. In view of the new and reliable evidence that "glutenin" in flour is not a definite chemical individual, and since cereal chemists in general have thus far found little or no practical value in existing methods for its estimation, the associate referee recommends² that further collaborative endeavors be discontinued.

¹ *J. Biol. Chem.*, 10, 15 (1911).

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 73 (1930).

REPORT ON HYDROGEN-ION DETERMINATIONS.

By C. H. BAILEY (University Farm, St. Paul, Minn.), *Associate Referee*.

It was recommended in 1928 that the Associate Referee on Hydrogen-ion Concentration give attention to the use of the quinhydrone electrode.

A flour sample was accordingly distributed, with the following instructions:

Determine the H-ion concentration of this sample during the period between September 4th and 9th, using both the *hydrogen*, and the *quinhydrone* electrodes, and report the results not later than September 10, 1929. Preparation of the flour extract for this purpose should be conducted as follows:

Weigh 10 grams of the flour into a 250 cc. flask and suspend in 100 cc. of distilled water at a temperature of 25°C. Maintain at 25°C. for 30 minutes, shaking at 5 minute intervals in order to keep the flour particles in suspension. Allow to stand exactly 10 minutes to permit the flour particles to settle, decant the supernatant liquid into the electrode vessel, and at once determine the H-ion concentration electrometrically.

For the quinhydrone electrode, either a gold wire or gold button is recommended. Five to ten cubic centimeters of the flour extract is sufficient, together with a slight excess of quinhydrone in order to effect saturation with this reagent. Allow 2-3 minutes to elapse after adding the quinhydrone, with frequent stirring during this period. The quinhydrone electrode is positive to the calomel half-cell, instead of negative as in the instance of the hydrogen electrode. In reporting, describe the characteristics of the half-cells, including the concentration of KCl solution in the calomel electrode vessel. Also please report results, both in terms of E. M. F. and in terms of pH, and supply the formulas (or cite the tables) used in converting the E. M. F. into terms of pH.

The results, as reported by the nine collaborators, appear in the attached tabulation in terms of pH. In general, the agreement secured by each collaborator was satisfactory with the possible exception of collaborator No. 6.

The variability is about the same in the instance of the two methods, although the number of reports is hardly adequate to the statistical treatment of the data. It accordingly appears that the quinhydrone electrode is suitable for use in this connection, although it may be well to caution prospective users of this method that in general the sensitivity of the potentiometer set-up is diminished when the ordinary quinhydrone electrode is substituted for the usual types of hydrogen electrodes.

It is recommended¹ that the present tentative method for the determination of hydrogen-ion concentration be amended to include a phrase stipulating that either the hydrogen electrode or the quinhydrone electrode may be used in the measurement of the pH of the extract prepared as² directed in the method.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 73 (1930).

Preliminary report of the collaborative pH determinations.

| COLLABORATIVE NUMBER | H-ELECTRODE pH | QUINHYDRONE ELECTRODE pH |
|-------------------------|-------------------|--------------------------------|
| 1 | 5.95 | 5.96 |
| 2 | 6.07 | ... |
| 3 | 6.08 | 6.05 |
| 4 | ... | 6.15 |
| 5 | 6.09 | 6.08 |
| 6 | 6.12 | 6.04 |
| 7 | 6.08 | 6.07 |
| 8 | 5.95 | 5.92 |
| 9 | 6.04 | 6.06 |
| Average of 8 | 6.05 | 6.04 |

REPORT ON DIASTATIC VALUE OF FLOUR.

By ARNOLD H. JOHNSON (Agricultural Experiment Station, Bozeman, Mont.), *Associate Referee.*

The preceding Associate Referee on Diastatic Value of Flour worked with the idea in mind of allowing the flour diastase to act on a substrate other than the starch in the flour containing the diastase, the purpose obviously being to distinguish between the activity of the diastase and the susceptibility of the substrate to diastasis. Glycogen appeared to be a suitable substrate, but that available always underwent some hydrolysis due to its contamination with diastase. His problem, therefore, was concerned with preparing a diastase-free glycogen.

Before continuing from this point the present associate referee decided to investigate certain factors influencing the diastatic activity which it is necessary to know. It is well known that the degree of fineness to which flour is ground has a marked influence on the diastatic activity. Thus, of two flours milled from the same wheat and of the same ash content, the flour containing the larger number of small particles will have the higher diastatic activity. It is further known that extraction of a flour with ether gives a flour of higher diastatic activity than that of the unextracted flour. Now, the extracted flour appears to be much finer than the unextracted flour, hence the apparent increase in diastatic activity may be attributed to increased susceptibility of the substrate to diastasis. It was thought that by extracting flours with ether, all flours would become of the same particle size, hence would provide a uniform substrate, and differences in sugar-producing power would be due entirely to differences in diastatic activity and not to differences in susceptibility of substrate to diastasis.

Two flours were milled from the same wheat, and one of them was reground until it had about twice the diastatic activity of the other. These two flours were then extracted with ether with the idea that this would produce flours of equal diastatic activity. This was not the case,

however. Both flours increased in apparent diastatic activity, but that of one was still only about half that of the other. This idea, therefore, had to be given up.

Another phase of the problem was also studied. The optimum hydrogen-ion concentration for the activity of flour diastase has received little attention. It is not known, for example, whether the optimum is the same in a third middlings flour as in a second clear flour. Two series of flours, each consisting of a third middlings, a straight, a first clear and a second clear, were therefore secured, and the optimum hydrogen-ion concentration for diastatic activity was determined. It was found that there existed a striking difference in the optimum. The third middling flours had their optimum around pH 3.5, while the second clear flours had their optimum around pH 5.0, with the other flours intermediate. Since there was the possibility of these differences being due to the different electrolyte content of the flours, the experiment was repeated, and the electrolyte contents were made comparable for all the flours. The results obtained, however, were the same. This phenomenon appears to complicate matters rather than otherwise, as the optimum hydrogen-ion concentration for the diastase in one flour may not be the optimum for that in another flour. Further work must be done on this phase of the problem¹.

REPORT ON STARCH IN FLOUR.

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The associate referee was directed to compare the Rask² method of determining starch in flour with a method that employs pepsin, as suggested in the method of determining total carbohydrates in cereal products by Hartmann and Hillig.

The Rask method was modified by filtering the starch into a Gooch crucible containing a considerable quantity of light fluffy ignited asbestos, drying, and weighing, then igniting and reweighing. The starch is represented by the loss in weight.

A method using pepsin to react on the proteins before determining starch was developed by first washing out the fat and sugars, as is done in the Rask method, and then following the procedure suggested by Hartmann and Hillig for the use of pepsin, the diastase and hydrochloric acid treatments, and finally making the copper reduction by the Munson and Walker method.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 74 (1930).

² *This Journal*, 11, 37 (1928).

Collaborative work was done by these two methods, but the results indicate that further work should be done before a decision on the merits of these methods is made. Therefore, it is recommended that further collaborative work be done¹.

REPORT ON FLOUR-BLEACHING CHEMICALS.

By G. C. SPENCER (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The collaborators' results for chlorine estimation in chlorine-bleached flour by the Seidenberg method² are presented. Two samples of commercially bleached flour were sent to the collaborators with nearly the same instructions as were furnished the previous year³. The samples were designated No. I and No. II, respectively.

No. I was a "Beta Chlora" bleached flour, while No. II was a flour which had been treated by the "Agene" process, which selection gave an opportunity for testing the Seidenberg method with flours of high and low chlorine content. Preliminary examination showed that Sample No. I was so high in added chlorine as to justify taking a 15 gram charge instead of the 20 grams which the original directions call for, and the collaborators were notified accordingly.

Sample No. I was prepared by blending equal weights of chlorine-bleached flour, treated with 1 oz., 1.5 oz., 2 oz., and 2.75 oz. of chlorine, respectively, per barrel. If these amounts of chlorine are actually absorbed and a perfect mixing is assumed, the resulting sample would contain 1.81 oz. of chlorine per barrel, or 577.1 parts per million of chlorine. The highest amount of chlorine reported by any collaborator is 295 p. p. m., or 51.1 per cent of the weight of chlorine applied by the "Beta Chlora" process.

Sample No. II, "Agene" bleached, was treated by the millers with 1.5 grams of nitrogen trichloride per barrel of flour. This corresponds to 16.9 p. p. m. of nitrogen chloride per barrel, or 14.9 p. p. m. of chlorine.

Published reports of chlorine found in untreated flours have indicated a chlorine content ranging from zero to 50 p. p. m. and even higher⁴. More recent results obtained with an untreated flour by the Seidenberg method, which is at present under consideration, have indicated that the amount of chlorine rarely runs higher than 20 p. p. m.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 74 (1930)

² *This Journal*, 11, 132 (1928).

³ *Ibid.*, 12, 391 (1929).

⁴ *Ibid.*, 6, 70 (1922); 7, 131 (1923).

The following results were obtained by the associate referee working with an unbleached flour and using the present Seidenberg method:

| | P. P. M. CHLORINE |
|------------------|-------------------|
| April, 1928..... | 0 |
| April, 1928..... | 0 |
| April, 1929..... | 24.9 |
| April, 1929..... | 7.6 |
| April, 1929..... | 8.6 |
| April, 1929..... | 16.7 |

TABLE 1.

Chlorine in bleached flour.

(Results expressed in p. p. m., water-free basis.)

| COLLABORATOR | 1 | 2 | 3 | 4 | 5 | 6 | 7 | MAX. | MIN. | AVE. |
|-------------------------------------|-----|-----|-----|-----|-----|-----|-----|---------|------|-------|
| FLOUR NO. I, "BETA CHLORA" PROCESS. | | | | | | | | | | |
| L. H. Bailey | 226 | 228 | | | | | | | | 227 |
| F. B. Carpenter, Jr. | 280 | 261 | | | | | | | | 270.5 |
| Emily Grewe | 287 | 281 | | | | | | | | 284 |
| O. F. Krumboltz | 267 | 270 | 268 | 295 | 289 | | | 295 | 267 | 277 |
| W. C. Luckow | 256 | 255 | 262 | 272 | 259 | 266 | 262 | 272 | 255 | 261 |
| G. C. Spencer | 246 | 236 | 272 | 252 | 272 | 285 | | 285 | 236 | 260.5 |
| Alice B. Vaughn | 237 | 251 | | | | | | | | 244 |
| R. Walker | 286 | 279 | 288 | 280 | | | | 288 | 279 | 283 |
| | | | | | | | | Maximum | | 284 |
| | | | | | | | | Minimum | | 227 |
| | | | | | | | | Mean | | 263 |

FLOUR NO. II, "AGENE" PROCESS

| | | | | | | | | | | |
|----------------------|-----|------|-----|-----|------|--|--|---------|-----|------|
| L. H. Bailey | 0 | 0 | | | | | | | | 0 |
| F. B. Carpenter, Jr. | 10 | 8 | | | | | | | | 9 |
| Emily Grewe | 0 | 0 | | | | | | | | 0 |
| O. F. Krumboltz | 1.1 | 2.2 | 8.0 | 5.7 | 11.4 | | | 8 | 1.1 | 5.6 |
| W. C. Luckow | 0.9 | 2.8 | 0 | 0 | 0 | | | | | 0.7 |
| G. C. Spencer | 4.1 | 3.5 | 7.8 | 8.5 | | | | 8.5 | 3.5 | 5.9 |
| Alice B. Vaughn | 6 | 8 | | | | | | | | 7.0 |
| R. Walker | 16 | 11.4 | 4.5 | 16 | | | | 16 | 4.5 | 11.9 |
| | | | | | | | | Maximum | | 11.9 |
| | | | | | | | | Minimum | | 0.0 |
| | | | | | | | | Mean | | 5.0 |

The following comments were made by some of the collaborators:

W. C. Luckow.—Under (f) in reagents, I believe it would be well to specify that the nitric acid used should be free from brown fumes.

Walker suggests that the charred residue in the platinum dish be warmed with nitric acid without boiling, and further that the directions be changed to read at this point, "Allow to cool and add 25 cc. of water", instead of "a small quantity of water".

RECOMMENDATIONS¹.

It is recommended—

(1) That the Seidenberg method for chlorine in bleached flour be adopted as a tentative method.

(2) That attention be directed during the coming year to possible methods for detecting added benzoic acid in flour.

REPORT ON FOREIGN METHODS FOR TESTING FLOUR.

By C. H. BAILEY (University Farm, St. Paul, Minn.), *Associate Referee*.

During the past few years considerable interest has been attracted to the limits in terms of acidity imposed by the Greek Government in the instance of flour offered for sale in that country. Previous to June 1, 1925, the requirement for low-grade flour was acidity not to exceed 0.25 per cent as sulfuric acid. A Greek Government protocol of December 30, 1924, provided, among other things, that second quality flour (ash content 0.50–1.0 per cent) shall contain a maximum acidity (as sulfuric acid) of 0.15 per cent. After considerable discussion of these standards they were placed in effect June 1, 1925.

The milling trade press announced in February, 1926, that these acidity restrictions had been removed, and there followed another announcement in Commerce Reports that the restrictions were reimposed in April, 1926. In August, 1926, the acidity limit of second-grade flour was apparently increased to 0.16 per cent, with a tolerance of 10 per cent thereof during the months of June, July and August. These limits are still in effect.

The imposition of restrictions of this type naturally attracted the attention of American cereal chemists to the determination of acidity in flour, in which there had been a minimum of interest as a routine analytical procedure. Much confusion arose in consequence of an effort to translate the results of acidity determinations by the A. O. A. C. method into terms of the Greek acidity method. In America the common practice, materializing in the A. O. A. C. tentative method², has

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 74 (1930).

² *Methods of Analysis*, A. O. A. C., 1925, 225.

been to titrate an aqueous extract of flour and express the results as lactic acid. In the instance of American clear grade flours, the acidity may exceed 0.40 per cent when thus determined. A direct conversion of this percentage as lactic acid gives 0.218 per cent as sulfuric acid. Or, in other words, a limit of 0.16 per cent as sulfuric acid would appear equivalent to about 0.30 per cent as lactic acid.

The error in such an assumption lies in the fact that the Greek Government chemists use 85 per cent alcohol as a solvent in the preparation of the flour extract for titration. Their procedure is essentially as follows:

Weigh 5 grams of flour into an 80 cc. flask provided with a ground-glass stopper and cover with 25 cc. of 85 per cent alcohol. Allow to digest for 24 hours with frequent agitation. Draw off 10 cc. of the supernatant liquid with a pipet and titrate with alcoholic 0.02 *N* sodium hydroxide, using tincture of curcuma as an indicator. The end point is a persistent "chamois" color. Each cc. of the standard alkali is equivalent to 0.00098 gram of sulfuric acid.

The acid-reacting, or more properly the alkali-neutralizing, constituents of such extract are not equal in quantity to those present in the aqueous extract prepared as in the tentative A. O. A. C. acidity method. This is evident from more than 80 comparative tests conducted by the Committee on Flour Specifications of the American Association of Cereal Chemists¹. What is even more disconcerting is the evident tendency toward a different ratio between results obtained by the two methods as the percentage of acidity changes from grade to grade, or with the lapse of time in the instance of any sample. An integration of the data from these 80 or more comparisons indicates a continuously changing rate of increase in the ratio as the acidity approaches zero. In other words, a unit increase in acidity in the lower levels effects a larger decrease in the ratio than a like increase in acidity in the higher levels. In a general way, the approximate ratios are of the following order when stated in terms of acidity by the Greek method:

| ACIDITY PERCENTAGE BY GREEK METHOD | MULTIPLY BY THIS FACTOR TO CONVERT INTO ACIDITY BY A. O. A. C. METHOD |
|---------------------------------------|---|
| 0.05 | 6.0 |
| 0.10 | 4.2 |
| 0.15 | 3.3 |
| 0.20 | 2.4 |

Despite these complications, the results obtained by either method serve as a reasonably adequate basis for estimating the probable percentage as determined by the other method. This is evident from the relatively large coefficient of correlation between the two sets of data obtained from the statistical analysis of 71 pairs of determinations, $r = 0.860 \pm 0.014$. The lowest coefficient of correlation appeared in the

¹ F. A. Collatz et al., *Cereal Chem.*, 7, 397 (1930).

instance of the spring wheat patent flours, the highest with the first clear durum wheat flours.

"SOUNDNESS" OF FLOUR.

At this stage of the studies of acidity methods, there is no basis for a conclusion as to which is best suited to the measurement of the relative "soundness" of flour. Soundness of flour is not exactly a tangible or definite property of such material, but is indeed the composite of characters which may relate to enzymic or other changes in the wheat used in its production, or to the single or joint action of flour enzymes or of fungi and other organisms in the flour after its manufacture. The regular march of flour acidity increase with the lapse of time, as determined by both methods, suggests that either may be employed to detect such changes.

Viewed from two angles at least, the use of alcohol as an extraction medium seems to offer distinct advantages over the extraction with water at 40°C. for 1 hour. In the latter procedure, time must be accurately controlled, since it appears that the apparent acidity tends to increase with time of extraction. With alcohol as the extraction medium, time is not a critical factor after half the usual extraction period has elapsed. This is the consequence of the practical inhibition of enzyme action in 85 per cent alcohol. In fact, the acidity of the 40°C. aqueous extract of flour may be regarded as the sum of the native acid-reacting substances plus the "acids" produced during the extraction period. The latter may constitute a considerable portion of the total, since the associate referee is inclined to believe that the relatively large difference in acidity between 40°C. and cold extracts is the consequence of fermentation changes rather than greater solubility in the warm water.

The second angle from which these methods may be viewed is that of the relative variability of results obtained with each, when applied by different analysts. Data at hand are not yet adequate to make a statistical analysis, but a casual survey of them indicates a tendency toward a greater variability in the instance of the A. O. A. C. method.

A survey of the literature indicates that alcohol is most commonly used in European acidity methods. The table represents an attempt to summarize certain of the methods that have been described. Several polemics have been conducted, notably those of Rammstedt (1913), Kreis and Arragon (1914), Fachinato (1908), and Pagniello (1908). Kalning (1919) has given a convenient summary of a number of the methods proposed for this purpose and reported the results obtained with a wheat flour and a rye flour subjected to test with eleven different methods. In general, aqueous extracts required more standard alkali to neutralize than alcoholic extracts.

Summary of acidity methods employed.

| REPORTED BY | DETAILS PUBLISHED BY | SOLVENT | CONDITIONS OF EXTRACTIONS | INDICATOR | ACIDITY EXPRESSED AS |
|--------------------------|--|---------------------------|---|-----------------|------------------------------|
| Arpin and Pecaud | <i>Ann. chim. anal.</i> , 4, 462 (1922) | 90% alcohol | Room temp. 24 hours | Curcuma | Sulfuric |
| Balland | <i>Compt. rend.</i> , 119, 565 (1894) | 85-90% alcohol | Room temp. 12 hours | Turmeric | Sulfuric |
| Planchon | Jago & Jago "Technology of Bread Making" (1911) | Water Water Alcohol | Room temp. 24 hours " | Phenolphthalein | Sulfuric |
| de Silvadon Seibra | <i>Rev. chim. pura applicada</i> , 1, 263 (1905) | Alcohol | | | Sulfuric |
| Dombrowsky | <i>Arch. Hyg.</i> , 50, 97 | Water | Room temp. | Phenolphthalein | Lactic |
| White | N. D. Agr. Exp. Sta. 20th Ann. Rept. (1909) | Water | Room temp. 2 hours | Phenolphthalein | Lactic |
| Swanson | Kans. Agr. Exp. Sta. Bull. 202 | Water | Room temp. 35 min. | " | " |
| Willard and Fitz Breteau | | Water | Room temp. 120 min. | | |
| | Falsifications et Alterations des Substances Alimentaires (p. 167) | 90% alcohol | Room temp. 12 hours | Litmus | Sulfuric |
| Girard | Analyse des Matières Alimentaires (p. 251) | 85% alcohol | Room temp. 24 hours | Curcuma | |
| Heiduschka and Deininger | <i>Z. Nahr. Genussm.</i> , 40, 161 (1920) | 90% alcohol | 20 min. on water bath evap. and dissolve in water | Phenolphthalein | |
| Hilger and Günther | <i>Mitt. Pharm. Erlangen II</i> , 13 (1889) | Absolute alcohol | In Soxhlet for 12 hours | Litmus | |
| Beythien | Handbuch (p. 393) 1913 | Absolute alcohol | In Soxhlet evap. and dissolve in water | Litmus | |
| Kreis and Arragon | <i>Schweiz. Wochschr.</i> , 38, 64 (1900-01) | Water | Boiled 30 min. | Phenolphthalein | Equivalent of N alkali |
| Schindler | <i>Z. landw. Versuchsw.</i> , 12, 79 (1909) | 85% alcohol | Room temp. 24 hours | " | Equivalent of 0.1 N alkali |
| Rammstedt | <i>Z. angew. Chem.</i> , 26, 677 | Absolute alcohol | Boil 30 minutes | " | Equivalent of N alkali |
| Kalning | <i>Z. ges. Getreidew.</i> , 11, 105 (1919) | Acetone | In extraction thimble 17 hours | " | Equivalent of N alkali |
| Lüers and Adler | <i>Z. Nahr. Genussm.</i> , 29, 281 (1915) | 96% alcohol | Heat at 70-80° for 30 minutes | | Equivalents of 0.1 N alkali |
| Prior | Brauer J., 4, 74 (1894) | Chloroform | 14 hours | | |
| Fachinato | <i>Gazz. chim. ital.</i> , 32, 543 (1902) | 85% alcohol | 24 hours | " | Sulfuric |
| Besley and Baston | U. S. Dept. Agr. Bull. 102 (1914) | 80% alcohol | 16-18 hours* Room temp. | | Equivalents of 0.01 N alkali |
| Birkner | <i>J. Agr. Research</i> , 18, 33 (1919) | Cold water | Ice-bath for 1.5 hours† | Phenolphthalein | Equivalents of 1.0 N alkali |
| Greek off. method | | 85% alcohol | Room temp. 24 hours | Curcuma | Sulfuric |

* Besley and Baston later, U. S. Dept. Agr. Circ. 68 (1916), substituted high-speed stirring for 30 minutes.

† Birkner extracted corn for 1.5 hours, oats for 1 hour.

F. A. Collatz, Chairman of the Committee on Flour Specifications of the American Association of Cereal Chemists, presented at the convention of that association in May, a discussion of the origin of the present tentative A. O. A. C. method as it appears printed in the Book of Methods (Chap. XVI, Sec. 5, 2nd ed., 1925, p. 225). He reported that the first mention of a method for the determination of flour acidity in the proceedings of the A. O. A. C. was made by E. F. Ladd, Associate Referee, in his report on Cereal Products in 1908¹. Ladd's colleague, H. L. White², reported on the determination of acidity of water extracts of flour by titrating an aqueous extract which was prepared by digesting 20 grams of flour for 2 hours with 200 cc. of water at 35° to 40°C. Phenolphthalein was used as indicator, and the results were expressed as lactic acid.

The White method differed in some details from the method suggested by Ladd (1908), who recommended the use of 18 grams of flour and 200 cc. of water, at 40°C., to be digested for 10 minutes at 40°C., and then allowed to stand 1 hour at room temperature before filtering and aliquoting for titration. Ladd reported that the acidity determined by digesting at 15°, 20° and 25°, respectively, tended to increase with the elevation of the temperature, and the method which he outlined is believed to give the maximum acidity.

In 1911 White as associate referee recommended two alternative methods for the determination of acidity: (a) Ladd's method (1908); and (b) the method that he had recorded previously².

In 1913 he³ recommended a combination of certain features of the Ladd method and of his methods that involved the extraction of 18 grams of flour with 200 cc. of water for 1 or 2 hours, filtration and titration of 100 cc. of the extract with 0.05 *N* sodium hydroxide, and the use of phenolphthalein as an indicator. It will be noted that the extraction period was not definitely fixed.

No further mention of flour acidity methods appears in the proceedings of the A. O. A. C. until 1915, when Committee C on Recommendations of Referees, H. E. Barnard, Chairman⁴, recommended the following method, with a view to its adoption as a provisional method, in 1916:

Weigh out 18 grams of flour into a 500 cc. Erlenmeyer flask and add 200 cc. distilled water free from carbon dioxide. Place flask in water oven kept at a temperature of 40°C. for 2 hours, shaking vigorously every half hour; filter through dry double filters, rejecting the first cc. of filtrate, until 100 cc. is obtained. Titrate with *N*/20 sodium hydroxid, using phenolphthalein as an indicator. Each cubic centimeter of sodium hydroxid solution represents 0.050 per cent of acidity in lactic acid.

It will be observed that this method calls for an extraction period of

¹ U. S. Dept. Agr. Bull. 122, p. 54.

² N. Dak. Agr. College 20th Annual Rpt. (1909).

³ *This Journal*, 1, 198 (1915).

⁴ *Ibid.*, 3, 87 (1917).

2 hours; otherwise the method is identical with that published in the A. O. A. C. *Book of Methods* (2nd ed., p. 225). In 1916, Referee LeClerc¹ reported the results of collaborative determinations of acidity, each collaborator employing four different methods, as follows: (a) Digesting for 2 hours at 40°C.; (b) digestion of flour for 1 hour at 40°C.; (c) water at 40°C. added to the flour and this mixture allowed to stand 1 hour at room temperature; and (d) water at room temperature added to the flour and allowed to stand for 2 hours at room temperature.

The differences in the results obtained by the four methods were not large, the averages of the results (acid as lactic acid) of nine collaborators being as follows: (a) 0.133 per cent; (b) 0.129 per cent; (c) 0.124 per cent; and (d) 0.121 per cent. The referee noted that Method C is the simplest.

It is interesting to observe the very wide variation in the results obtained with any one method by the nine collaborators. Thus, with Method A the range was from 0.095 per cent to 0.172 per cent; and with Method C it was from 0.084 per cent to 0.160 per cent.

The brief reference to the acidity determination in LeClerc's report of 1916 constitutes the last reference to this determination which the present associate referee has been able to locate in the proceedings of the association, with the following exception found on page 452 of the same volume:

(3) That Method (c) for acidity be approved, and that the methods for the determination of acidity in flour receive further study.

In the mind of the reader, this leaves the official status of the A. O. A. C. method for flour acidity in a very confused state. There is no evidence that the recommendation of Subcommittee C in 1915 was adopted or acted upon in 1917 or at any subsequent time. Moreover, the recommendations of the referee in 1917² are not referred to in the recommendations of Committee C of the same year³, so the recommendation by the referee for the approval of acidity Method C was not carried to the association in Committee C's recommendation.

In view of this fact, it was interesting to note that in the first edition of the A. O. A. C. *Official and Tentative Methods of Analysis* (1920) and also in the second edition (1925) the tentative acidity method calls for aqueous extraction for 1 hour at 40°C., a procedure which is neither the recommendation of Subcommittee C in 1915, or of Referee LeClerc in 1916.

Hertwig⁴, in 1925, suggested that the most acceptable manner of expressing titratable acidity of flours would be in terms of cubic centi-

¹ *This Journal*, 449 (1920).

² *This Journal*, 3, 452 (1920).

³ *Ibid.*, 532.

⁴ *This Journal*, 9, 389 (1926).

meters of 0.1 *N* alkali per 100 grams of sample. The phrasing of Subcommittee C's recommendations in Section (17) of their report¹ makes it appear that this revision of the acidity method was not recommended for study in 1926.

In practically all these studies of the acidity method by referees and associate referees of the A. O. A. C., seemingly no serious consideration has been given to the European practice of extracting flour with alcohol. The associate referee accordingly recommends² that the collaborative study of the acidity method be reopened, with a view toward (a) ascertaining the fundamental sources of variability in the application of the tentative A. O. A. C. method, and (b) the comparison of these results with those obtained by an alcoholic extraction procedure essentially similar to the present Greek acidity method.

REPORT ON SAMPLING AND DETERMINATION OF MOISTURE IN BREAD.

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

It was shown last year that moisture in a one pound loaf of pan bread was not uniformly distributed throughout the loaf. This year the work was continued with loaves of different sizes and shapes.

Four loaves, representing that many types of bread, were used for this experiment; they were commercial loaves obtained from bakery stands in the market and were said to be fresh bread.

The loaves were first weighed and then divided into four parts by cutting through lengthwise and crosswise. The four portions were not always of equal weight, due in part to their irregular shape. After each of the four portions had been weighed it was sliced in thin slices, spread on paper on the laboratory tables and allowed to dry until completely air dried, after which each portion was weighed, ground, and placed in an air-tight bottle. Moisture determinations were made on the air-dried material, and from all the data obtained moisture in the original loaf was calculated.

Loaf I was called a "home-made" loaf; it was $5\frac{1}{2} \times 8\frac{1}{2} \times 4$ inches and weighed $1\frac{1}{2}$ pounds. The four portions were designated A, B, C, and D.

| | FRESH WEIGHTS | AIR DRY WEIGHTS | MOISTURE IN— | |
|-----|---------------|-----------------|------------------|-------------------|
| | | | AIR DRY PORTIONS | ORIGINAL PORTIONS |
| | <i>grams</i> | <i>grams</i> | <i>per cent</i> | <i>per cent</i> |
| A = | 165 | 118 | 10.86 | 36.24 |
| B = | 160 | 114 | 10.79 | 36.44 |
| C = | 149 | 107 | 10.62 | 35.82 |
| D = | 165 | 117 | 10.76 | 36.76 |

¹ *This Journal*, 9, 90 (1926).

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 74 (1930).

There was a maximum variation of 0.94 per cent of moisture in the different portions of this loaf. The moisture in the whole loaf was 36.36 per cent.

Loaf II was a one pound rye loaf, hearth baked. The weights and percentages were as follows:

| | FRESH WEIGHTS | AIR DRY WEIGHTS | MOISTURE IN— | |
|-----|---------------|-----------------|------------------------------|-------------------------------|
| | grams | grams | AIR DRY PORTIONS per cent | ORIGINAL PORTIONS per cent |
| A = | 99 | 74 | 11.06 | 33.53 |
| B = | 142 | 104 | 10.96 | 34.79 |
| C = | 121 | 90 | 10.91 | 33.74 |
| D = | 115 | 84 | 10.96 | 34.96 |

In this sample the maximum variation in moisture in the different portions was 1.43 per cent. The moisture in the whole loaf was 34.30 per cent.

Loaf III was a one pound Vienna loaf, hearth baked. The weights and percentages were as follows:

| | FRESH WEIGHTS | AIR DRY WEIGHTS | MOISTURE IN— | |
|-----|---------------|-----------------|------------------------------|-------------------------------|
| | grams | grams | AIR DRY PORTIONS per cent | ORIGINAL PORTIONS per cent |
| A = | 125 | 96 | 10.59 | 31.35 |
| B = | 114 | 86 | 10.67 | 32.62 |
| C = | 104 | 79 | 10.71 | 32.17 |
| D = | 122 | 93 | 10.66 | 31.90 |

The maximum variation in moisture in the different portions of this loaf was 1.27 per cent. The moisture in the whole loaf was 31.98 per cent.

Loaf IV was an ordinary one pound loaf of pan bread. Its weights and percentages follow:

| | FRESH WEIGHTS | AIR DRY WEIGHTS | MOISTURE IN— | |
|-----|---------------|-----------------|------------------------------|-------------------------------|
| | grams | grams | AIR DRY PORTIONS per cent | ORIGINAL PORTIONS per cent |
| A = | 109 | 78 | 10.70 | 36.10 |
| B = | 130 | 92 | 10.71 | 36.82 |
| C = | 96 | 68 | 10.65 | 36.70 |
| D = | 130 | 93 | 10.72 | 36.13 |

In this loaf the maximum variation in moisture was 0.72 per cent. The moisture in the whole loaf was 36.44 per cent.

Even in this last loaf, which was the most symmetrical of the four, the maximum variation was 0.72 per cent, which is entirely too large to permit taking any portion of the loaf for a sample for the determination of moisture. These observations confirm those made last year. Therefore it is recommended that an entire loaf of bread be taken as a sample for the determination of moisture.

Assuming that the entire loss of weight during drying is due to moisture, this value (moisture) may be obtained by subtracting the percentage of total solids from 100.

It is recommended that the tentative method for the determination of total solids in an entire loaf of bread¹ be made official (final action)².

No report on lipoids and fat in bread was given by the associate referee.

No report on milk solids in milk bread was given by the associate referee.

No report on organic and ammoniacal nitrogen in air-dried baked cereal products was given by the associate referee.

REPORT ON CRUDE FIBER IN ALIMENTARY PASTES AND IN AIR-DRIED BAKED CEREAL PRODUCTS.

By W. F. STERLING (Food and Drug Administration, Washington, D. C.), *Associate Referee*.

In order to test the applicability of the official method for the determination of crude fiber in flour to baked cereal products and alimentary pastes, this determination was made on three samples of bran wafers and a sample of spaghetti. The following results, expressed in percentage of crude fiber, were obtained:

| | | | | |
|---------------------------|-------------|-------------|-------------|-----------|
| Material..... | Bran Wafers | Bran Wafers | Bran Wafers | Spaghetti |
| Sample No..... | 1 | 2 | 3 | |
| Crude Fiber (per cent)... | 2.99-2.93 | 5.67-5.75 | 3.42-3.47 | 0.42-0.38 |

No mechanical difficulties whatever were manifested during any of the determinations. The technic of the method used for flour appears to be entirely satisfactory and gave acceptable results in duplicate determinations on the products under consideration.

Therefore, it is recommended³—

(1) That the official method for the determination of crude fiber in flour be made official for air-dried baked cereal products (final action).

(2) That the official method for the determination of crude fiber in flour be made official for alimentary pastes (final action).

No report on rye flour in rye bread was given by the associate referee.

¹ *This Journal*, 9, 42 (1926).

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 74 (1930).

³ For report of Subcommittee C and action of the association, see *This Journal*, 13, 75 (1930).

REPORT ON EXPERIMENTAL BAKING TESTS.

By M. J. BLISH (Agricultural Experiment Station, Lincoln, Neb.),
Associate Referee.

During the past year the tentative standard experimental baking test has been subjected to collaborative study. The first and more extensive series of collaborative tests was a prominent feature of the activities of the committee on standardization of the Experimental Baking Test of the American Association of Cereal Chemists, on which the writer served. This committee also conducted preliminary investigations on items such as types of ovens, effect of different methods of molding the dough upon bread characteristics, system of reporting results of test bakes, accuracy of commercial dough thermometers, construction and greasing of baking pans, calibration of loaf volume measuring apparatus, and yeast testing. A full report of the committee's activities has been published by its chairman, C. G. Harrel¹.

These tests involved the efforts of 29 collaborators, some of whom were more experienced than others in the use of the standard test. There was a conspicuous lack of uniformity among the bread characteristics of the different collaborators, nor was this discordance of results confined to the less experienced operators. The results clearly and conclusively indicated a lack of uniformity as to the environmental conditions under which the tests were performed by the respective collaborators. Among those environmental factors that appeared to contribute the most heavily to the general discordance of the collaborative results are the following:

1. Unsatisfactory specifications and means for controlling oven temperatures especially with miscellaneous types of baking ovens.
2. Individual variations in molding and panning the doughs.
3. Variations in yeast.

Other factors, such as greasing or non-greasing of pans, errors in loaf volume measuring devices, lack of precise and reliable control of fermentation boxes, etc., doubtless played a minor, although significant part. The tests were highly informative in emphasizing the complexities and difficulties that are inevitably involved in any effort to standardize a biochemical procedure of this character.

Following this series of tests the associate referee undertook a series in which the selection of collaborators was confined to a few of its more experienced users. Six collaborators participated in the later series, and their results constitute the basis of the present report.

Two flours, a baker's and a family patent, were selected, and a sample of each was sent to the collaborators, respectively. They were requested

¹ *Cereal Chem.*, 6, 249-312 (1929).

to bake each of the two flours, in triplicate, by the basic procedure¹. Each flour was then to be baked, in triplicate, with the addition of 1 mg. of potassium bromate to the dough, according to Supplement C of the standard method. The collaborative results were reported by the method recently suggested², and these reports, together with a specimen of the proposed report form, are herewith presented. The reference models used for reporting external loaf characteristics are as published previously³. The reference models for internal characteristics have been superseded by a later set of photographic models, and the later set, as published in a more recent issue of the same publication⁴, was used in the present report. The baker's patent flour is designated as number 1, and the family flour as number 2. Plus signs indicate the same samples baked with the addition of 1 mg. of potassium bromate, according to Supplementary Method C.

The data, and more especially the photographs, readily reveal the nature and extent of variation among the collaborative results. Collaborator D was obviously the farthest out of line. This collaborator reported the doughs to be so soft and sticky at the specified absorption that he had to reduce the amount of water to the extent of 5 or 6 per cent. Two collaborators reported that the doughs were soft, but they were able to use the specified absorption or very nearly that. The other three made no comments indicating unusual slackness of dough. No explanation for the trouble experienced by collaborator D is at present available.

Variations among crust colors are believed to be due mainly to differences in oven conditions. The recent findings of Harrel, previously quoted in this report, readily justified this assumption. In the matter of loaf volume, the degree of concordance among the different collaborators is, in the opinion of the writer, excellent. In several instances corresponding loaves of different operators check as closely in volume as might be expected of duplicates baked by the same individual.

Several collaborators agree fairly well as to external conformation of the loaf, as is more apparent from the photographs than from the tabular data, minor points of disagreement being doubtless due to variations in molding and, in lesser degree, to oven conditions.

There is a fairly satisfactory agreement as to the *nature* of the differential between loaves baked with and without the 1 mg. of potassium bromate, to the extent that nearly all treated loaves show a decrease in volume. Any lack of agreement here is more in *degree* than in *kind*.

From the standpoint of progress that may be expected in the general project of standardizing the laboratory baking test, it is of interest to

¹ *Cereal Chem.*, 6, 249 (1929).

² *Ibid.*, 258.

³ *Ibid.*, 5, 292 (1928).

⁴ *Ibid.*, 6, 255 (1929).

TABLE 1.
Baking test report of Collaborator A*.

| SAMPLE NO. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
|-------------------|---------------------------|-------|-------|---------------|-------|-------|-------|-------|--------|---------|-------|-------|--------|---------|-------|----------|
| Basic test | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Supplementary | | | | | | | | | | | | | | | | |
| Moisture (%) | 12.75 | | | | | | | | 12.75 | | | | | | | C, 1 mg. |
| Flour used (gm.) | 97.4 | | | | | | | | 97.4 | | | | | | | |
| Water used (cc.) | 60.6 | | | | | | | | 60.6 | | | | | | | |
| Loaf volume (cc.) | 450 | 440 | 445 | 440 | 430 | 430 | 420 | 420 | 420 | 430 | 430 | 440 | 440 | 405 | 395 | 395 |
| Loaf weight (gm.) | 134.3 | 134.9 | 135.1 | 135.4 | 135.4 | 135.4 | 135.6 | 135.6 | 134.1 | 134.6 | 134.6 | 134.0 | 135.4 | 135.6 | 135.3 | 135.3 |
| External | J-Hs | | | J-Hs | J-Hs | J-Hs | | | J-Hs | | | | J | | | |
| Internal | 3 | | | 4 | | | | | 2+ | | | | 2 | | | |
| Firmness of crumb | M+ | | | S | | | | | M | | | | | | | |
| Texture | M | | | M | | | | | M | | | | | | | |
| Crust color | M | | | M | | | | | M to P | | | | | | | |
| Crust mottling | - | | | + | | | | | + | | | | ++ | | | |
| Crumb color | Cr, somewhat muddy & dull | | | Cr, less dull | | | | | somewh | at dull | | | somewh | at dull | | |
| Remarks | | | | | | | | | | | | | | | | |

* KEY TO TABLES: *External*.—A single letter, as F, G, or H, etc., denotes resemblance to one of the standard types or models. A combination of two letters, i. e., FG, GF, GH, etc., denotes combined characteristics of two types, the predominating type indicated by the first letter. The small letters, s, m, or l, may be added, if desired (as F_s, G_m, etc.), to indicate that the break is small, medium or large, as compared with the type model.

Internal.—Resemblance to type is reported by number. Numbers 1 to 5, inclusive, denote pronounced gradations in size of grain for round cell structure only. Elongated cell-structure may be indicated by adding the letter "E". Intermediate gradations are denoted by the use of plus or minus signs. Thus, 2+ means grain finer than 2 but coarser than 3. Numbers 6 and 7 are special types, 6 denoting the fluffy thin walled cells, while 7 represents an irregular, distorted structure. Numbers 6 and 7, respectively, may be combined with any one of the other numbers, to indicate both size and structure. Thus, "6-3" denotes the fairly large grain of 3, but with the thin cell walls of 6, while "7-2" denotes the large grain of 2 combined with the distorted appearance of 7.

Firmness of crumb.....S (strong), M (medium), or W (weak).

Texture.....H (hard), M (medium), or S (soft).

Crust color.....By letter or number, or D (dark), M (medium), or P (pale).

Crust mottling.....By plus or minus signs, i. e., +, ++, +++, etc.

Crumb color.....Wh, Cr, Y, etc.

TABLE 2.
Baking test report of Collaborator B.

| SAMPLE NO. | 1 | 1 | 1 | 1+ | 1+ | 1+ |
|-------------------|-------|-------|-------|----------|----------|----------|
| Basic test | ✓ | ✓ | ✓ | | | |
| Supplementary | | | | C, 1 mg. | C, 1 mg. | C, 1 mg. |
| Moisture (%) | 13.28 | | | | | |
| Flour used (gm.) | 98.3 | | | | | |
| Water used (cc.) | 60 | | | | | |
| Loaf volume (cc.) | 482 | 477 | 485 | 458 | 465 | 445 |
| Loaf weight (gm.) | 132.5 | 133.1 | 133.3 | 133.7 | 133.0 | 132.4 |
| External | G-Hm | Fm | F-Gm | G-Hm | | G-Hs |
| Internal | 4E | 3+ | | 6-4 | | |
| Firmness of crumb | S | S | | M | | |
| Texture | M | | | M | | |
| Crust color | M | | . . . | M | | |
| Crust mottling | + | | | ++ | | |
| Crumb color | Cr Wh | | . . . | | | |
| Remarks | | | | | | |

TABLE 3.
Baking test report of Collaborator C.

| SAMPLE NO. | 1 | 1 | 1 | 1+ | 1+ | 1+ |
|-------------------|-------|------|------|----------|----------|----------|
| Basic test | ✓ | ✓ | ✓ | | | |
| Supplementary | | | | C, 1 mg. | C, 1 mg. | C, 1 mg. |
| Moisture (%) | 11.65 | | | | 11.55 | |
| Flour used (gm.) | 96.2 | | | | 96.1 | |
| Water used (cc.) | 58 | | | | 58 | |
| Loaf volume (cc.) | 445 | 440 | 440 | 420 | 425 | 435 |
| Loaf weight (gm.) | 131 | 134 | 135 | 133 | 131 | 132 |
| External | G | | F | J | Hs | |
| Internal | 4+ | | | 2+ | 3 | |
| Firmness of crumb | S | | | | S | |
| Texture | S | | | M | M | |
| Crust color | M | | | | M | |
| Crust mottling | ++ | | | - | ++ | +++ |
| Crumb color | Cr | | | | Y | |
| Remarks | | | | | | |

TABLE 4.
Baking test report of Collaborator D.

[illegible]

TABLE 5.
Baking test report of Collaborator E.

| SAMPLE NO. | 1 | 1 | 1 | 1+ | 1+ | 1+ |
|-------------------|-------|------|------|----------|----------|----------|
| Basic test | ✓ | ✓ | ✓ | | | |
| Supplementary | | | | C, 1 mg. | C, 1 mg. | C, 1 mg. |
| Moisture (%) | 12.50 | | | | | |
| Flour used (gm.) | 98.2 | | | | | |
| Water used (cc.) | 59.8 | | | | | |
| Loaf volume (cc.) | 420 | 420 | 415 | 385 | 400 | 430 |
| Loaf weight (gm.) | 134 | 134 | 137 | 134 | 134 | 133 |
| External | Fm | Fs | Fm | | Fm | Fm |
| Internal | 4+ | | | | | 4+ |
| Firmness of crumb | M | | | | | |
| Texture | M | | | | | |
| Crust color | M | | | | | |
| Crust mottling | + | | | | + | + |
| Crumb color | Cr Wh | | | | | Gr-Wh |
| Remarks | | | | | | |

TABLE 6.
Baking test report of Collaborator F.

| SAMPLE NO. | 1 | 1 | 1 | 1+ | 1+ | 1+ |
|-------------------|-------|-------|-------|----------|----------|-------|
| Basic test | ✓ | ✓ | ✓ | | | |
| Supplementary | | | | C, 1 mg. | C, 1 mg. | |
| Moisture (%) | 13.30 | | ... | | | |
| Flour used (gm.) | 97.3 | | | | | |
| Water used (cc.) | 59.7 | | | | | |
| Loaf volume (cc.) | 480 | 480 | 470 | 405 | 410 | 400 |
| Loaf weight (gm.) | 131.2 | 134.5 | 130.3 | 130.6 | 130.7 | 132.4 |
| External | F-G | | | J | | |
| Internal | 3 | . . | | 2 | | |
| Firmness of crumb | S | | | | | |
| Texture | M | .. | | | | |
| Crust Color | M | | ... | M- | | |
| Crust mottling | + | ... | .. | ++ | | |
| Crumb color | Wh | . | . . | Gr | . . | . . |
| Remarks | | | | | | |

compare the present series of collaborative studies with the previous and more extensive series of tests conducted by Harrel¹. There is far more encouraging concordance of results in the present than in the former series. This, however, is only to be expected, since the present series involved fewer collaborators and was restricted to those presumed to have had extensive experience in its use. There had also been afforded the opportunity to contemplate the findings of the previous series and to gain a better appreciation of possible and probable causes for the enormous variation among the results of the first extensive collaborative tests.

Improvements in the uniformity of collaborative test baking results may be anticipated only at the same rate at which cereal chemists improve their facilities for securing precise, accurate and uniform control of all environmental factors. The time should eventually arrive when all factors with the possible exceptions of yeast and atmospheric pressure will be under satisfactory and uniform laboratory control. The factors of mixing, molding, ovens and yeast are probably the more prominent contributors to the difficulties that are being encountered under present circumstances.

The associate referee feels that definite and substantial progress has been made on a project that involves extraordinary difficulties and complications. He recommends² that the Association of Official Agricultural Chemists continue its cooperative efforts with the American Association of Cereal Chemists toward the establishment of a standard and official experimental baking test, and that the work be carried forward on its present basis.

It is admittedly difficult to portray an accurate picture of loaf characteristics by the use of descriptive terms, and this handicap is felt more strongly in the initial stages of the project than will be the case when greater general familiarity with the test is gained, and when the proposed reporting system has been improved, as it doubtless will be. In this series, therefore, the collaborators were asked to send a representative loaf from each set of bakes, by parcel post, to the associate referee. These loaves were then photographed, both as to external and internal characteristics. Each collaborator mailed to the associate referee a representative loaf from each bake, and all of these loaves were photographed. Although not reproduced in this report, these photographs are in the possession of the associate referee, and they are available to any who may be interested.

¹ Loc. Cit.

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 75 (1930).

REPORT ON WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, ASH AND TOTAL SOLIDS IN FLOUR AND ALIMENTARY PASTES.

By SAMUEL ALFEND (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*.

At last year's meeting it was recommended that a report be made by the referee on the tentative acid hydrolysis method for fat and the tentative methods for lipoids and lipoid phosphoric acid in alimentary pastes, recommended as official (first action) in 1927. These methods have been checked by thorough collaborative study and they have been used, without objection since 1927, in the associate referee's laboratory and elsewhere. They are therefore considered worthy of adoption as official methods (final action).

It was recommended that further collaborative work be done with the tentative F. A. C. method for the determination of unsaponifiable matter in the fat of alimentary pastes, baked cereal products and flour. This method has been studied collaboratively for three years, with no appreciable improvement in the uniformity of results obtained. It appears to be of little use to do further collaborative work on the method until it has been studied thoroughly by the associate referee, with a view to determining what factors are causing the irregularities in results. The associate referee has been unable to conduct such a study this year owing to lack of time.

The results obtained by the collaborators on alcohol-precipitable nitrogen have never been entirely satisfactory, and results obtained last year and the comments of the collaborators indicated the need for a thorough study of the method. Through an oversight, last year's directions omitted the addition of sodium chloride before the alcohol precipitation in one modification. This omission was noted by several collaborators, who reported results obtained with and without salt. In at least one case, the omission of salt resulted in a higher nitrogen value in the egg noodle, but the same method applied to liquid and dried eggs failed to produce a precipitate in some cases and gave a smaller quantity of precipitate in others.

The associate referee investigated the effect of sodium chloride in flour and alimentary pastes, as well as in eggs. Adding the alcohol before the salt instead of after was found to result in higher values in some instances, but to have no effect in others. When salt was omitted entirely, the precipitate formed more slowly, but it gave as high results, after it was allowed to coagulate overnight, as the determinations made with salt. In some cases the results were distinctly higher when salt was omitted.

The rise in temperature caused by the addition of the alcohol appeared to have no influence on the formation of the precipitate, as samples

run at temperatures varying from 15° to 35°C. gave approximately the same results.

It was difficult to obtain good check results, partly because of the difficulty in packing the precipitate on centrifugalizing, partly because of the frequent formation of a colloidal solution which passed through the filter pad. An important source of variation appears to exist in the extraction of the water-soluble protein from the sample. When several different samples of noodles or of flour were extracted with water under the same conditions it was not unusual for one to filter fairly clear while another gave an almost milky filtrate.

After working with the method continuously for several weeks, the associate referee came to the conclusion that the present method is not reliable for all samples. A decided improvement was the use of a 1.2 per cent solution of sodium chloride to extract the proteins from the sample, since, as Palmer showed¹, the albumin diffuses readily into a 1.2 per cent salt solution to form a fairly clear solution. It is significant that the best collaborative results on record for this determination were obtained in 1927, when one of the methods tested called for the use of 1.2 per cent salt solution for extraction². Work along this line was discontinued, however, because this method yielded higher results, and all the authentic data available are based on water-soluble nitrogen.

As one of the chief difficulties seemed to be the packing of the alcohol precipitate, and the subsequent filtration, an attempt was made to find a substance which would cause a good pack. Asbestos was found to be practically useless, because it separated from the precipitate when centrifugalized and settled at the bottom, the precipitate remaining above it. Calcium carbonate behaved in a similar manner. Alumina cream gave an excellent pack from which the clear liquid could be poured off readily. The results obtained, however, were much higher than those obtained without its use. This might be due to adsorption of alcohol-soluble protein, which subsequent washing failed to remove, or it might be due to the carrying down of finely suspended matter which came through the filter pad in the water extraction. Colloidal matter might be carried down by mutual precipitation, adsorption, or mechanical inclusion. The logical step was to add alumina cream to the aqueous extract just before filtration from the flour or noodles. This procedure gave a somewhat clearer solution for flour and noodles, but in the case of powdered whole egg and liquid egg it changed a muddy filtrate into a sparkling clear solution. The difference between the quantities of alcohol-precipitable nitrogen in the solutions before and after clarification with alumina cream was striking. The results for the clarified solu-

¹ *This Journal*, 8, 615 (1925).

² *Ibid.*, 11, 490 (1928).

tions were lower than the usual values, particularly in the case of the various egg samples.

It is possible that a revision in the method, which would apply to eggs as well as to flour and noodles, would necessitate the partial abandonment of the present data on alcohol-precipitable nitrogen in eggs and flour, and the gathering of new data. This, perhaps, is made less undesirable by the fact that such data as are available at present on egg products and flour are extremely meager, and not particularly concordant. At any rate, it is felt that more study is necessary and that the method finally adopted as official should give concordant results on any samples of flour, noodles, or the various types of eggs.

Some work has been done in this laboratory on the methods of analysis of bread recommended for further study, but insufficient collaborative work has been done to warrant further recommendations.

RECOMMENDATIONS¹.

It is recommended—

(1) That study of the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour and alimentary pastes be continued.

(2) That the method for the determination of unsaponifiable matter in flour and alimentary pastes be further studied before it is submitted for collaborative work.

(3) That work be continued on methods for the determination of fats, lipoids, and unsaponifiable matter in bread.

No report on collecting and preparing sample of alimentary paste for analysis was given by the associate referee.

No report on moisture in alimentary pastes was given by the associate referee.

H. B. McDonnell: You are honored in having present the representative of the United States Department of Agriculture, the honorable Assistant Secretary of Agriculture, Mr. Dunlap, and I am sure he needs no introduction to this audience. We are glad to hear from Mr. Dunlap.

ADDRESS BY R. W. DUNLAP, ASSISTANT SECRETARY OF AGRICULTURE.

MR. CHAIRMAN, LADIES AND GENTLEMEN: I know by your program that you expected to hear at this time from the Secretary of Agriculture, and I am sure you will be disappointed when the Assistant Secretary

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 71 (1930).

appears in his stead. I may be able to fill his shoes, but I am sure that I cannot fill his hat. I regret, as you do, that owing to the rush of problems and many engagements, the Secretary is not able to be here. In these days, however, we all do some substituting. The label is often false or misleading, as it appears to be in this case. Your program calls for a Secretary, and the Assistant Secretary appears.

I am not an entire stranger to you men; that is, to most of you. I see men in this audience with whom I worked a long time ago—about 20 years ago—in the food and drug work. I was at one time a food official of the State of Ohio, and I have kept more or less in touch with many of the food officials throughout the United States and have watched the progress which has been made in food and drug law and enforcement.

I did not come here this afternoon to make a speech. I do not profess to be a scientific man. Strange to say, I am a farmer. Of course it may be surprising to some of you to know that there is a farmer in the Department of Agriculture, but it so happens that there is in the case of the present speaker. It is not necessary that I extend to you a welcome. We are all welcome to our own, and this city and the Department of Agriculture are yours.

You are here, I understand, as the offspring of the Department of Agriculture, for I am told that about 49 years ago this organization sprang up because of the interest that was taken by certain individuals in the Department, and you are back here at this time for a family reunion, now a bigger and stronger association. I assume you are having some fun while here. I do not begrudge you all the fun you can have, for I am sure that we are much too serious as we go through life. You are here in this family reunion to discuss matters pertaining to your business and you certainly are welcome, not only to this great city but to the Department as well. I believe this is your forty-fifth convention. Consequently, this organization must be worth while. The organizations that do not amount to anything soon disappear, but the fact that you are continuing is ample evidence that you really are accomplishing something.

I am one of those who is of the opinion that the scientific men and women of this country will in time solve most of our farm problems. There are many laws being enacted in the legislatures of our various States and in our Congress for the purpose of helping agriculture. These laws may help temporarily. Your work and discoveries are permanent and not of doubtful value. As an example, you are working on the problem of producing a concentrated fertilizer. I don't know whether you realize it or not, but I know that if you can give us a fertilizer that will contain as much plant food in one ton as we now get in three or four tons, it is going to be worth millions and perhaps billions of dollars to the farmers of this country, and I am certain you will succeed. This is

just one illustration of the way you are going to help solve the farm problem. I might cite many more.

I said I did not come here to make a speech, and I am going to sign off right now. I just want you to know that over in the Department of Agriculture we are interested in you, and we are interested in what you are doing. We are interested in your association, and I am speaking for the whole Department when I say that we want to continue the very friendly relations that have existed during the past. If at any time we can be of help to you in your respective States, I am sure that all you will have to do will be to call upon us. I sincerely hope that you have had a pleasant and profitable meeting.

No report on beers, wines and distilled liquors was given by the referee.

No report on specific gravity and alcohol was given by the referee.

REPORT ON VINEGAR.

By ARTHUR M. HENRY (U. S. Food and Drug Administration, Philadelphia, Pa.), *Referee*.

In part compliance with the recommendation of the referee for the last two years, methods for the determination of ash and glycerol and for polarization were studied. No work on the methods for the determination of phosphoric acid and sulfates was done. The referee wishes to express his appreciation to Jacob Fitelson and Paul A. Mills of the Philadelphia Station, who did the analytical work.

TOTAL ASH.

Total ash was determined in the referee's laboratory on samples of vinegar and vinegar stock by Method 5(b)¹ and by the same method with the addition of 1 gram of commercial sucrose. The results obtained by J. Fitelson, analyst, expressed as grams per 100 cc., are given in Table 1.

These determinations were made in an ordinary electric muffle, without automatic temperature control, which was kept at just visible red. It was noted that there was a difference in color in the various parts of the muffle. The differences in ash by the two methods may be accounted for by difference in temperature in different parts of the muffle. However, the addition of sugar reduces the number of water treatments,

¹ *Methods of Analysis*, A. O. A. C., 1925, 325.

TABLE 1.
Collaborative Results.

| PRODUCT | ASH | | NO. OF TIMES WATER ADDED | | TIME REQUIRED, HOURS | | CHARACTER OF CHANGED VINEGAR | | APPEARANCE OF FINAL ASH | |
|---------------------|------------------|------------------------|-----------------------------|------------------------|-------------------------|------------------------|---------------------------------|------------------------|-------------------------|------------------------|
| | 5(b) | 5(b) + 1 g. SUCROSE | 5(b) | 5(b) + 1 g. SUCROSE | 5(b) | 5(b) + 1 g. SUCROSE | 5(b) | 5(b) + 1 g. SUCROSE | 5(b) | 5(b) + 1 g. SUCROSE |
| Cider Stock, No. 1 | 0.390 | 0.411 | 2 | 2 | 6.0 | 2.0 | not puffy | not puffy | brown | brown white |
| " " No. 2 | 0.356 | 0.360 | 2 | 1 | 6.0 | 1.0 | " | slightly puffy | " | " |
| " " No. 3 | 0.326 | 0.332 | 2 | 2 | 5.5 | 4.5 | " | puffy | " | " |
| " " No. 4 | 0.314 | 0.350 | 2 | 2 | 7.0 | 3.5 | " | " | white | white |
| Cider Vinegar No. 1 | 0.406 | 0.421 | 2 | 1 | 6.0 | 1.0 | " | very puffy | brown | brown white |
| " " No. 2 | 0.312 | 0.300 | 2 | 1 | 5.5 | 2.0 | " | puffy | brown white | " |
| " " No. 3 | 0.298 | 0.302 | 2 | 2 | 8.5 | 2.5 | puffy | very puffy | white | white |
| " " No. 4 | 0.272 | 0.284 | 2 | 1 | 7.0 | 1.5 | " | puffy | " | " |
| " " No. 5 | 0.259 | 0.255 | 2 | 1 | 7.0 | 1.5 | " | very puffy | brown white | brown white |
| Distilled Vinegar | 0.054 | 0.058 | 0 | 0 | 3.5 | 1.0 | not puffy | puffy | grey brown | grey white |
| Ave. Sugar 1 g. | | not weigh- able | | 0 | | 1.0 | | very puffy | | white |
| Molasses Vinegar | 0.819 | 0.856 | 1 | 0 | 5.5 | 1.0 | | puffy | | |
| | +0.012 -0.057 | +0.057 -0.012 | Maximum variation | | | | | | | |
| | -0.013 | +0.013 | Average variation | | | | | | | |

reduces the length of heat treatment, and improves the character of the charred residue and final ash. The ash from the 1 gram of sugar alone is not weighable.

POLARIZATION.

Considerable work was done in the referee's laboratory on samples of various kinds of vinegar by Fitelson and Mills. Readings were made on a 200 mm. tube at 20°C. on the vinegars without other treatment than filtration; with the addition of 10 cc. of alumina cream¹ to 50 cc. of the vinegar and correction of reading for dilution; with the addition of 5 cc. of saturated neutral lead acetate solution to 50 cc. of the vinegar; removal of the excess of lead by anhydrous potassium oxalate and correction of the reading for dilution; and treatment of the vinegar with four different commercial vegetable decolorizing carbons as given in the tentative method². The results, expressed in °V., are given in Table 2.

TABLE 2.

Polarization of vinegar treated with various decolorizing agents.

| PRODUCT | NONE | ALUMINA CREAM— 10 CC. | NORMAL LEAD ACETATE REMOVED WITH POTASSIUM OXALATE | TREATMENT WITH VEGETABLE CARBONS | | | |
|--|----------|-----------------------------|--|-------------------------------------|----------------------------|----------------------------|-------------------------|
| | | | | A | B | C | D |
| Cider Vinegar No. 1 | -1.8 | -1.8 | -1.7 | -1.8 | -1.6 | -2.2 | -1.7 |
| " " No. 2 | -1.8 | -1.8 | -1.9 | -1.5 | -1.4 | -1.1 | -1.4 |
| " " No. 3 | -1.5 | -1.4 | -1.5 | -0.9 | -1.3 | -1.1 | -0.9 |
| " " No. 4 | -0.6 | -0.6 | -0.6 | -1.3 | -1.0 | -1.0 | -0.6 |
| " " No. 5 | -0.9 | -1.0 | -0.9 | -0.6 | -0.4 | -0.5 | -0.6 |
| " " No. 6 | -0.6 | -0.6 | -0.6 | -0.3 | -0.4 | -0.4 | -0.1 |
| Distilled Vinegar, No. 1 | +0.1 | +0.1 | +0.2 | +0.1 | +0.0 | +0.1 | +0.1 |
| " " No. 2 | +0.1 | +0.1 | +0.1 | +0.1 | +0.2 | +0.4 | +0.2 |
| Molasses Vinegar | Too dark | Too dark | -0.3 | -0.1 | -0.2 | -0.3 | -0.1 |
| Variation from un- treated vinegar | | -0.1 to +0.1 | -0.1 to +0.1 | -0.7 to +0.6 =1.3 | -0.4 to +0.5 =0.9 | -0.4 to +0.7 =1.1 | 0 to +0.6 =0.6 |
| Average variation from untreated vinegar | | 0.00 | +0.01 | -0.01 | +0.16 | +0.17 | +0.29 |

¹ *Methods of Analysis*, A. O. A. C., 1925, 182.

² *Ibid.*, 329, par. 23.

Alumina cream is not a good clarifying agent, because it fails to remove the colloidal suspended matter, although it does lighten the color very well.

Normal lead acetate, with removal of the excess lead, does not remove all the color in some vinegars, but in all determinations enough color was removed for satisfactory readings. The vegetable decolorizing carbons are the best for removing the color and clarifying the solutions, as it is possible to obtain a water-white solution in all cases, although in some cases two or three treatments were necessary. However, the carbons not only remove the color but also the odor and flavor of the vinegar and leave an odor of ethyl acetate. Determination of the acidity before and after treatment with carbon showed a small loss in all cases.

It appears from Table 2 that alumina cream and neutral lead acetate, with removal of the excess lead, have very little, if any, effect on the polarization of vinegars as shown by comparison of readings before and after treatment. The variations are all within the limits of experimental error. The use of carbon shows wide variations and a tendency to give a positive error. From this work it appears that neutral lead acetate with removal of excess lead is the best method. No vinegars with considerable amounts of non-volatile acid or with an excess dextrose over levulose were available for study. Further study should be given this subject before any procedure is recommended for adoption as the official method.

GLYCEROL.

Much work has been done on various phases of this method. The results obtained show the need for further work. A 1 per cent solution of phenylamine in concentrated sulfuric acid has been used satisfactorily as an inside indicator instead of potassium ferricyanide as an outside indicator in the final titration. The work has not progressed far enough to recommend any changes at this time.

RECOMMENDATIONS¹.

It is recommended—

(1) That methods for the determination of total and soluble ash be further studied, with particular attention paid to the use of sucrose or other substance for reducing the time of heating and to the temperature of ashing.

(2) That in connection with the study on ash the methods for the determination of phosphoric acid be further studied.

(3) That the method for the determination of glycerol be further studied.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 76 (1930).

(4) That the tentative method for the determination of sulfates be submitted to collaborative study with a view to adoption as official.

(5) That the method for polarization be further studied.

(6) That methods 14 and 15 for the determination of reducing substances be rewritten so as to require the use of "dilute hydrochloric acid (sp. gr. 1.1029 at 20°/4°C. or 24.8–24.9° Brix at 20°C.)", instead of "strong hydrochloric acid", so as to be uniform with other methods for reducing substances.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES.

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.), *Referee*.

In accordance with the suggestion made last year by Subcommittee C, additional collaborative work was done on the method for the determination of citral¹ in lemon and orange oils and extracts which was tested last year. The results obtained are set forth in Table 1.

TABLE 1.

CITRAL IN LEMON EXTRACT (COLORIMETRIC METHOD)

| SAMPLE* | J. I. Palmore per cent | P. A. Clifford per cent |
|---------|---------------------------|----------------------------|
| 1 | 0.14 | 0.15 |
| 2 | 0.23 | 0.26 |
| 3 | 0.15 | 0.17 |
| 4 | 0.22 | 0.24 |
| 5 | 0.20 | 0.22 |

* As these samples are the same as those examined last year, the results obtained are comparable with those in Table 1 of last year's report².

The results of the two collaborators agree very well, and they also agree well with the results reported last year on the same samples. One of the collaborators this year had had experience with the method and the other had not. In view of the consistently satisfactory results obtained last year and this year with the modified method, it is the opinion of the referee that it should now be made official, final action.

In accord with one of the recommendations made by the referee last year, a method for the determination of total aldehydes in lemon and orange oils and extracts was tested in comparison with the official Kleber method³. The new method was devised by Radcliffe and Swann; it depends upon the addition of a known amount of pure thiosemicarbazide to 2 or 3 grams of oil or 50 cc. of extract to form the thiosemicarbazone. The unacted-upon thiosemicarbazide remaining after removal of the citral thiosemicarbazone and of certain oil constituents with a

¹ *This Journal*, 12, 48 (1929).

² *Ibid.*, 404.

³ *Methods of Analysis*, A. O. A. C., 1925, 355.

suitable solvent is weighed in pure form. The weight of aldehyde is calculated from the reacting quantity of the reagent. The outstanding advantages of the method are its applicability to both essential oils and extracts, the ease and rapidity with which it can be carried out, and the small quantity of sample needed. A description of the method follows:

TOTAL ALDEHYDES.

REAGENTS.

- (a) *Thiosemicarbazide crystals* (m. p. 180° – 182° C.).
- (b) *Alcohol*.
- (c) *Carbon disulfide*.

DETERMINATION.

Weigh accurately from 2 to 3 grams of oil (or about 50 cc. of extract) and about 0.8 gram of thiosemicarbazide into a 200 cc. Erlenmeyer flask fitted into a reflux condenser. Add 50 cc. of alcohol (the addition of alcohol is not necessary in the case of an extract) and reflux on the steam bath. When all or nearly all the thiosemicarbazide is in solution, detach from the reflux condenser and evaporate to dryness on the steam bath. Remove the last traces of alcohol by passing a current of air into the flask while it is immersed in boiling water.

Add 150 cc. of pure carbon disulfide and boil on the steam bath under the reflux for a few minutes to dissolve the thiosemicarbazone and the non-aldehydic constituents of the oil. Cool, filter through a tared filter, transfer the undissolved thiosemicarbazide to the filter, and wash well with carbon disulfide. Dry the filter and contents to constant weight at 50° C. Subtract the weight of thiosemicarbazide on the filter paper from the amount used and multiply by 1.87 to obtain the weight of total aldehydes expressed as citral.

The results obtained on three lemon oils by three collaborators by the above method and by the Kleber method are given in Table 2.

TABLE 2.

Determination of total aldehydes in lemon oil.

| SAMPLE* | J. B. WILSON | | C. H. BADGER | | W. O. WINKLER | |
|-------------|------------------------|---------------------------|------------------------|---------------------------|------------------------|---------------------------|
| | New method per cent | Kleber method per cent | New method per cent | Kleber method per cent | New method per cent | Kleber method per cent |
| F. C. 589-B | 3.20 | 3.01 | 3.19 | 2.89 | 3.05 | 2.83 |
| | 3.19 | | 3.36 | 3.01 | 3.08 | 2.73 |
| | 3.21 | | 3.26 | | | |
| | | | 3.60 | | | |
| F. C. 590-B | 4.23 | 3.69 | 4.17 | 3.87 | 3.80 | 3.48 |
| | 3.91 | | 4.04 | 3.95 | 3.68 | 3.47 |
| | 4.21 | | | 3.97 | 3.75 | |
| F. C. 591-B | 4.17 | 3.86 | 4.13 | 4.04 | 4.09 | 3.60 |
| | 3.96 | | 3.92 | 4.15 | 3.95 | 3.59 |
| | 4.24 | | | 4.07 | | |

* F. C. 589-B was California oil, 6 years old; F. C. 590-B was hand-pressed Italian oil, 6 years old; and F. C. 591-B was hand-pressed Italian oil, 1 year old.

DISCUSSION OF DATA IN TABLE 2.

Of the 24 results recorded in Table 2, 23 obtained by the new method are higher than the average results obtained by the Kleber method, the

average difference being 0.26 per cent. Moreover, the differences vary greatly, that is, from -0.06 to $+0.71$. The alcohol reagent had a blank of 0.04 per cent, which would reduce the average difference to 0.22. Radcliffe and Swann did not compare their method with the Kleber method, but they did compare it with the hydroxylamine method, the official method of the British Pharmacopeia, and obtained much better checks than those recorded in Table 2. It is believed that collaborators should obtain results by any new method for the determination of total aldehydes in lemon oil that agree within 0.2 per cent of the amount of aldehydes actually present, the best criterion of which at the present time is believed to be the official Kleber method, before the association would be warranted in adopting such a method as an alternative official method.

The results obtained by the new method are such as to preclude its adoption until further collaborative work is conducted. The referee believes that the method is worthy of further study and that it is possible to make certain modifications to improve it. For example, weighing the thiosemicarbazide on a tared filter is disadvantageous because it is difficult to obtain a constant weight. In addition to a further study of this method, search should be continued for other methods that appear worthy of investigation and that are applicable both to oils and extracts, as is the method tested this year.

RECOMMENDATIONS¹.

It is recommended—

(1) That the present official method for the determination of citral in lemon and orange oils and/or extracts² be dropped (final action).

(2) That the method for the determination of citral in lemon and orange oils and/or extracts³ published previously be adopted as official (final action).

(3) That the official Kleber method⁴ be removed from its place under the heading "Lemon and Orange Oils—Citral" and placed under the heading "Lemon and Orange Oils—Total Aldehydes" and that its official character be retained (see last year's report).

(4) That more extensive collaborative work be done on the gravimetric method described in this year's referee report, or modifications of it, for the determination of total aldehydes in orange and lemon oils and/or extracts, and that the search be continued for other methods for total aldehydes that are applicable to both oils and extracts.

(5) That the study of the steam distillation method for the analysis of non-alcoholic flavors be discontinued for the present.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 77 (1930).

² *Methods of Analysis*, A. O. A. C., 1925, 354.

³ *This Journal*, 12, 48 (1929).

⁴ *Methods of Analysis*, A. O. A. C., 1925, 355.

REPORT ON MEAT AND MEAT PRODUCTS.

By R. H. KERR (Bureau of Animal Industry, Washington, D. C.),
Referee.

No cooperative work was carried on during the year. Following the adoption last year of the method for the estimation of added water in meat and meat products, consideration was given to the development of a more rapid method for the determination of total moisture. Considerable work was done by H. R. McMillin in the Washington Meat Inspection Laboratory of the Bureau of Animal Industry. He made tentative studies of two methods: one a distillation method involving the use of a solvent having a boiling point somewhat higher than that of water, and one involving air drying at a temperature well above 100°C. The latter proposal may seem to be somewhat radical, but it may be pointed out that fresh meat and other animal tissues contain but little, if any, substance known to be decomposed by heat at temperatures below 150°C., and that in the commercial rendering of fats, temperatures up to 135°C. are commonly employed.

In commercial operations the nitrogeous constituents, being of the highest value, are usually most carefully checked by chemical analysis, and it is generally known that the nitrogen contained in the fresh charge subjected to the ordinary rendering operations may be quite accurately accounted for by analysis of the rendered material. This fact in itself is an indication that no great decomposition takes place. Heating to 125°C. as an analytical procedure, therefore, would appear to prove satisfactory.

McMillin's experiments indicate that if proper precautions are taken, total moisture in meats and meat products can be determined by drying at 125°C. with an accuracy comparable with that of the official method and within a time period of from 2 to 3 hours. For the purpose of analytical control, this means that a report can be made the same day on samples delivered to the laboratory by noon. It is the intention of the referee to have a method for the rapid determination of total moisture in meats and meat products by drying in air at 125°C. ready for cooperative study during the ensuing year¹.

No report on separation of meat proteins was given by the associate referee.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 69 (1930).

REPORT ON GELATIN.

By R. M. MEHURIN (Bureau of Animal Industry, Washington, D. C.),
Referee.

The last published report¹ made by the retiring referee states that unsatisfactory results were obtained in collaborative work on the determination of copper and zinc in gelatin by the employment of the tentative methods of analysis. It has been the experience of the present referee that uniform results cannot always be expected when these methods are employed by the average analyst, unless he has had some previous experience with their use. With a view to meeting this condition, a simplified method involving ashing of the sample and the colorimetric estimation of copper was devised and submitted, with two samples of gelatin, to the two collaborators who signified their willingness to cooperate in this work. They were requested to compare this method with the alternative method², which, like the tentative method, involves hydrolysis of the sample. The results reported, however, are not considered sufficiently satisfactory to warrant a detailed report of the experiments and the publication of the method at this time. It may be stated, however, that collaborators obtained more uniform results by use of the proposed method than by use of the alternative method. The following comments were made by collaborators:

W. D. Richardson, Swift & Co.—1.—Proposed method preferred. It is simple, quick, and gives closer results.

2.—Sample taken as directed under alternative method was too small. In one case 1 mg. makes a difference of 40 p. p. m. and in the other case 20 p. p. m.

C. R. McKee, U. S. Gelatine Co.—1.—The color standards described in the proposed method are unstable and must be matched quickly.

2.—The alternative method presented many difficulties on account of the amount of organic matter present, which was complicated by the addition of iron and the formation of colloidal iron in the presence of organic matter; 20 cc. of hot, dilute nitric acid was not sufficient to dissolve the sulfides. Formic acid and ammonium thiocyanate may cause the filtrate containing the zinc to become turbid, thus making necessary repeated filtrations.

The referee has obtained excellent results in the past with a method for the determination of copper and zinc in gelatin similar to that sent to collaborators; he is of the opinion that the preliminary ashing of the sample and colorimetric estimation of copper will result in considerable saving of time and, in the hands of the average chemist, give greater accuracy in results than is obtained by hydrolysis of the sample and gravimetric or volumetric estimation of copper.

It is recommended³ that further comparative study be made of the ashing and hydrolysis methods for the determination of copper and zinc in gelatin.

¹ *This Journal*, 9, 458 (1926).

² *Methods of Analysis*, A. O. A. C., 1925, 256.

³ For report of Subcommittee C and action of the association, see *This Journal*, 13, 78 (1930).

REPORT ON SPICES AND OTHER CONDIMENTS.

By KENNETH C. BEESON (State Chemical Laboratory, Vermilion, S. D.),
Referee.

A comparative study of the present tentative method for the determination of lecithin phosphoric acid in salad dressings¹, the method proposed by Andrew², and a method outlined by the referee has been started. It was found that regardless of what method of extraction is used the determined value for egg yolk and the calculated value (obtained by determining lecithin phosphoric acid in the eggs used) check very closely if the method of determining the lecithin phosphoric acid in the salad dressing is the same as that used for the egg yolk. Naturally, therefore, any method proposed must be accompanied by the value of lecithin phosphoric acid in the eggs determined by the proposed method. More time will be required to study these points before any methods are proposed or any collaborative work is requested.

The tentative method for reducing sugars³ is not entirely satisfactory owing to the impossibility of breaking up the oil emulsion in some cases simply by extracting the sample of mayonnaise with petroleum ether. For this reason the following modified method is offered for collaborative study:

REDUCING SUGARS BEFORE INVERSION—PROPOSED METHOD.

Add to 20 grams of the sample in a wide-mouthed 4 ounce bottle, 10 cc. of alcohol (95 per cent) and 2 cc. of strong ammonium hydroxide. Heat the mixture to boiling on the steam bath, cool, and extract the oil by adding about 80 cc. of petroleum ether, shaking, and centrifugalizing. Decant as much of the petroleum ether solution as possible, and repeat the treatment with petroleum ether until all the oil has been removed. This condition is indicated by the absence of color in the solvent. Usually about four extractions are required. Reserve the ether solution for identification of the oil. Remove the petroleum ether from the residue with a current of air and transfer the residue with water to a 100 cc. volumetric flask. Neutralize the mixture in the flask with hydrochloric acid. Continue the determination as directed in *Methods of Analysis*, 321, par. 37, beginning with "Add 5-10 cc. of a fresh solution of metaphosphoric acid".

RECOMMENDATIONS⁴.

It is recommended—

(1) That the study of the lecithin phosphoric acid determination be continued.

(2) That the method proposed for reducing sugars before inversion be studied collaboratively along with the present tentative methods⁵ for total solids, oil, reducing sugars after inversion, and total acid.

¹ *Methods of Analysis*, A. O. A. C., 1925, 322.

² *This Journal*, 8, 700 (1925).

³ *Ibid.*, 321.

⁴ For report of Subcommittee C and action of the association, see *This Journal*, 13, 78 (1930).

⁵ *Methods of Analysis*, A. O. A. C., 1925, 320.

REPORT ON CACAO PRODUCTS.

By J. O. CLARKE (U. S. Food and Drug Administration, Chicago, Ill.),
Referee.

Crude Fiber.—The associate referee continued an investigation of a modified crude fiber method. The present tentative method is quite satisfactory for cacao material such as cocoa or bitter liquor where the factor for converting to the fat- and moisture-free basis is comparatively small. With sweet chocolate and milk chocolate, the total fat-free cacao mass is of the order of 10 per cent, and therefore the conversion factor to water-, sugar- and fat-free basis is about 10. Any allowable error in the actual fiber as determined is multiplied by that percentage. What is more important is that the factor is based on the actual determination of fat, sugar and water, and any error in these figures is reflected to a magnified extent in the fiber on the water-, sugar- and fat-free basis.

The crude fiber figure on cacao products is worthless unless referred to the moisture-, fat- and sugar-free basis or to some comparable basis, for the reason that in the manufacture of these products widely variable amounts of sugar and fat are incorporated in the mixture. On the basis of the entire sample, therefore, the crude fiber figure has no meaning.

When the results obtained in 1925 by 11 collaborators on a sweet chocolate¹ were calculated to the moisture-, sugar- and fat-free basis, the following results were obtained:

| MOISTURE | SUCROSE | FAT | CRUDE FIBER | |
|-----------------|-----------------|-----------------|-----------------|--|
| | | | As determined | Calculated to fat-water-sugar-free basis |
| <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 0.30 | 56.59 | 37.04 | 0.31 | 5.11 |
| 0.49 | 58.05 | 37.08 | 0.45 | 10.27 |
| 0.33 | 59.28 | 36.91 | 0.23 | 6.61 |
| 0.20 | 57.82 | 37.05 | 0.30 | 6.09 |
| 0.41 | | 36.18 | 0.62 | |
| 0.25 | 59.54 | 35.98 | 0.51 | 12.06 |
| 0.29 | 57.01 | | 0.81 | |
| 0.25 | 57.60 | 37.28 | 0.72 | 14.78 |
| 0.26 | 58.91 | 36.99 | 0.52 | 13.54 |
| 0.17 | 57.49 | 37.04 | 0.42 | 7.92 |
| 0.32 | 58.65 | 37.30 | 0.59 | 15.82 |

These results vary from 5.11 to 15.82 and show the extreme inaccuracy of the present tentative method. An error of 1 per cent each in sucrose and fat makes an error of 2 per cent in the non-fat cacao mass. The average non-fat cocoa mass in the above sample was 7.26 per cent. Therefore, on this assumption this figure might be 9.26 per cent or 5.26

¹ *This Journal*, 9, 461 (1926).

per cent. The average crude fiber on the above sample was 0.49. Converting to the fat- and sugar-free basis, the moisture not being important here, the referee got 9.32 per cent or 5.29 per cent, depending on an allowable error in sugar and fat.

The associate referee has shown in previous reports that a comparatively accurate figure, yielding fairly good collaborative results, can be obtained by determining the crude fiber on a sample weighed out from the cocoa mass after water, ether and alcohol-soluble material have been extracted and dried. This figure, however, is not the crude fiber of authentic samples of cacao products on which a mass of authentic data have been accumulated, the difference being due to the water-soluble cacao material extracted in the preparation of the sample. On the theory that this water-alcohol-soluble material is fairly constant, the arbitrary figure obtained by the proposed method can be converted to the usual basis. Preliminary results indicate that the water-alcohol-soluble material is fairly constant and is in the neighborhood of 30 per cent. Even if the figure is somewhat variable, the proposed method will yield more concordant results than the present tentative method.

Cacao Butter.—The associate referee studied the qualitative test for coconut and palm kernel oils and showed by collaborative work that the method is capable of yielding accurate results. He proposes a slight modification in the procedure which will tend to simplify the method.

Milk Solids and Sucrose.—No work was done.

RECOMMENDATIONS¹.

The referee approves the recommendation of the Associate Referee on Crude Fiber and Cacao Fat.

The methods for the determination of sucrose and milk solids should be studied.

REPORT ON CRUDE FIBER IN CACAO PRODUCTS.

By MARIE L. OFFUTT (Food and Drug Administration, New York, N. Y.), *Associate Referee*.

Last year's collaborative results², obtained by the proposed method for the determination of crude fiber in cacao products, showed that this method gave higher figures than those obtained by the tentative method upon which the standard was based. Because these higher figures were due to the amount of material washed out by the water and alcohol, it seemed necessary to determine this amount in order to convert the arbitrary figure of the proposed method to the formal standard one by a constant factor.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 78 (1930).

² *This Journal*, 12, 419 (1929).

This amount was determined by weighing the ether-extracted dry residue, which was then washed with water, alcohol and ether according to the method; this dry residue was weighed, and the amount of water and alcohol-soluble material in the ether-dry residue was calculated. The results are given in Table 1.

TABLE 1.

Water and alcohol-soluble material (F) in ether-dry residue.

| SAMPLE | per cent |
|--------|----------------------|
| 1 | 28.23 |
| 2 | 29.81 |
| 3 | 29.34 |
| 4 | 29.81 |
| 5 | 29.21 |
| 6 | 30.37 |
| 7 | 30.26 |
| 8 | 29.88 |
| 9 | 30.20 average — 29.7 |

The percentage of water and alcohol-soluble material in the ether-dry residue (*F*) appears to be fairly constant on the samples examined. The effect of different temperatures of water used in washing was tried on one sample, with the following results:

Room temperature around—

| °C. | °F. |
|-----|-------|
| 30 | 30.37 |
| 28 | 29.75 |
| 25 | 28.57 |
| 18 | 29.59 |
| 12 | 28.92 |

As the temperature variations do not seem to have any decided effect, room temperature appears convenient for use.

The method proposed in previous reports is given below with certain modifications:

Treat 7 grams of liquor or 50 grams of sweet chocolate with 100 cc. of ether in a nursing bottle, centrifuge, and decant the supernatant liquor twice; dry the residue in an oven at about 100°C. and then powder in the bottle with a flattened glass rod. In some cases it may be found necessary to grind the material in a mortar and extract a third time with ether. Wash in the nursing bottle with three 100 cc. portions of distilled water at room temperature, shaking well each time until no cocoa material adheres to the bottle. Centrifuge after each washing for 10–15 minutes and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc. portions of 95 per cent alcohol and one 100 cc. portion of ethyl ether. Transfer the residue to a platinum dish, dry to constant weight, and grind in a mortar. Weigh 2 grams of the dried material and determine crude fiber by the usual A. O. A. C. method. Report results as the percentage of crude fiber in the washed and dried material.

Convert the figure for crude fiber (*D*) to the formal standard one (*E*) by using the factor *F* found in Table 1 by following the equation—

$E = D - (D \times F)$, in which

D = the percentage of crude fiber in washed and dried material;

F = the percentage of water- and alcohol-soluble material in ether-dried residue;
and

E = the percentage of crude fiber on moisture-, fat- and sugar-free basis.

It will be noted that the amount of liquor taken in the proposed method has been changed from 15 grams to 7 grams, as the latter was found to give the 2 grams required for the crude fiber determination—more than 50 grams of sweet chocolate may be required in some cases to give the 2 grams.

Table 2 gives the figures on 9 samples of bitter chocolate and 9 samples of sweet chocolate by the proposed method and also these figures converted to the moisture-, fat- and sugar-free basis, the F obtained on each being used,—that is, the arbitrary figure called $D - (D \times F)$ equals E , the fiber on moisture-, fat-free basis. On some of the samples E had been run by the tentative method, and the figure so obtained is also given. On the sweet chocolates, the average (F) of Table 1, obtained on bitter chocolates, is used to calculate E from D .

The numbers in brackets used with the samples of sweet chocolate indicate that the sweet chocolate was made from the liquor of the respective number above. The results show close agreement.

TABLE 2.

Results obtained with bitter and sweet chocolate.

| SAMPLE | D | E (CALCULATED) | E (BY TENTATIVE METHOD) |
|------------------|-------|-------------------|----------------------------|
| BITTER CHOCOLATE | | | |
| 1 | 8.05 | 5.78 | 6.81 |
| 2 | 9.14 | 6.42 | 6.85 |
| 3 | 9.21 | 6.51 | 7.14 |
| 4 | 9.71 | 6.82 | 6.97 |
| 5 | 8.19 | 5.80 | 6.18 |
| 6 | 9.80 | 6.83 | 6.74 |
| 7 | 9.08 | 6.33 | |
| 8 | 9.05 | 6.35 | |
| 9 | 8.40 | 5.86 | |
| SWEET CHOCOLATE | | | |
| A (6) | 10.34 | 7.27 | 7.59 |
| B (1) | 8.54 | 6.00 | |
| C (2) | 8.94 | 6.28 | |
| D (3) | 9.53 | 6.70 | 6.14 |
| E (3) | 9.59 | 6.75 | |
| F (5) | 8.55 | 6.01 | |
| H (5) | 7.98 | 5.61 | |
| G (4) | 9.32 | 6.55 | |
| L (4) | 9.47 | 6.66 | |

While collaborators obtained close results on the fiber on the original basis, that figure when calculated to the moisture-, fat- and sugar-free basis varied decidedly; for example, a variation of 0.43 on the original

basis on a sample when changed over was 3.26, due to slight difference in sugar and fat percentages found by the different collaborators.

Table 3 shows the variation of E calculated from D by F when F is varied or both D and F are varied and the highest and lowest results are taken. The variation found by collaborators with the proposed method last year was less than by the tentative; so if a constant factor is used to convert the arbitrary figure to the formal one, more uniform results should be obtained.

TABLE 3.
Variation of E .

| D | | F | E (CALCULATED) | |
|-----------------|------|-----------------|------------------|------|
| <i>per cent</i> | | <i>per cent</i> | <i>per cent</i> | |
| 9.80 | | 30.37 | | 6.82 |
| 9.80 | | 29.59 | | 6.90 |
| 9.80 | | 28.57 | | 7.00 |
| 9.80 | | 28.92 | | 6.97 |
| 9.80 | | 29.75 | | 6.88 |
| | Max. | 30.37 | | 6.82 |
| | Min. | 28.57 | | 7.00 |
| Diff. | | 1.80 | Plus | 0.18 |

| D | | F | E (CALCULATED) | |
|-----------------|-------|-----------------|------------------|------|
| <i>per cent</i> | | <i>per cent</i> | <i>per cent</i> | |
| | 10.25 | 30.37 | | 7.19 |
| | 10.25 | 28.57 | | 7.32 |
| | 9.28 | 30.37 | | 6.46 |
| | 9.28 | 28.57 | | 6.63 |
| High | 10.25 | 28.57 | | 7.32 |
| Low | 9.28 | 30.37 | | 6.46 |
| Difference | | | Minus | 0.86 |

The F found and used here applies only to bitter and sweet chocolates as it was necessary to use a different method¹ for milk chocolates, as reported last year. The F for milk chocolate could be determined the same way as F here reported, or the F already found might be used with some factor determined for the amount of milk not washed out. Work was started along this line, but it could not be completed in time for this report.

RECOMMENDATIONS².

It is recommended—

(1) That the modified proposed method for bitter and sweet chocolates be studied further and collaborative work done.

(2) That the associate referee study the method with a view to making it applicable to milk cacao products.

¹ Lepper-Waterman, *J. Ind. Eng. Chem.*, 19, 501 (1927).

² For report of Subcommittee C and action of the association, see *This Journal*, 13, 78 (1930).

REPORT ON CACAO BUTTER.

By MARION M. JACKSON (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*.

This year, as a continuation of the study of foreign fats in cacao butter, the qualitative test for the detection of coconut and palm kernel oils was subjected to further examination. The underlying principle of the method is confined to the turbidity formed when the potassium soaps of caproic, caprylic, and capric acids, which remain in solution after being salted out twice, are acidified. Since both palm kernel and coconut oils contain more of the glycerides of these acids than butter fat, they will cause a heavier turbidity. Obviously, a blank containing butter fat is necessary when milk chocolates are to be analyzed.

Two known, A and B, and six unknown samples, C to F, inclusive, were sent to five collaborators, all of whom are members of the U. S. Food and Drug Administration, for analysis by this test.

As A and B were designated as blanks, A being pure cacao butter and B, 90 per cent cacao butter and 10 per cent butter fat, they are reported negatively, though one of the analysts obtained a slight turbidity in B. The other reports on A and B indicate clear solutions for the final products. C, containing cacao butter and palm kernel oil, was declared doubtful by two and positive by three of the analysts. D and F, containing coconut oil, were reported positive by all the analysts, and H, which was pure cacao butter, was declared negative by all. E, containing cacao butter, milk fat and palm kernel oil, was reported positive by four analysts and negative by one analyst, and G, which had the same composition as B, was declared faintly positive by one analyst, positive by another and negative by the other three analysts.

These results compare favorably with those obtained previously on this test, and they indicate that the method is quite satisfactory. However, the standard that is to be used when milk chocolates are to be analyzed may be simplified.

By the present method, a blank of cacao butter containing milk fat in equal proportion to that present in the cacao butter to be analyzed is made up. This procedure, obviously, necessitates preparing a different blank for each sample. Now, the percentage of milk fat in cacao butter necessary to produce a very faint opalescence is about 15 per cent, and as the butter fat in the cocoa butter of milk chocolate usually runs 15 per cent or less, a blank of 85 per cent cocoa butter and 15 per cent butter fat ought to fulfill all the requirements for the test for cacao butter containing 15 per cent or less of milk fat. If changed in this way, the method would be greatly shortened and more helpful to the analyst.

Collaborative results.

| ANALYST | SAMPLE A | SAMPLE B | SAMPLE C | SAMPLE D | SAMPLE E | SAMPLE F | SAMPLE G | SAMPLE H |
|--|----------------------|-----------------------------------|--|------------------------------------|---|---|---------------------------------|----------------------|
| P. A. Clifford Washington, D. C. | Pure cacao butter | 90 % cac. but. 10 % milk fat | 90 % cac. but. 10 % palm kern. oil | 90 % cac. but. 10 % coconut oil | 80 % cac. but. 10 % milk fat 10 % palm kern. oil | 80 % cac. but. 10 % milk fat 10 % coconut oil | 90 % cac. but. 10 % but. fat | Pure cacao butter |
| N. E. Freeman Chicago, Ill. | Negative | Negative | Positive | Positive | Positive | Positive | Negative | Negative |
| H. W. Haynes Boston, Mass. | Negative | Negative | Doubtful | Positive | Positive | Positive | Negative | Negative |
| F. O. Kellems San Francisco, Calif. | Negative | Negative (slight turbidity) | Positive | Positive | Positive | Positive | Positive | Negative |
| M. L. Offutt New York, N. Y. | Negative | Negative | Positive | Positive | Negative | Positive | Faintly positive | Negative |

RECOMMENDATIONS¹.

It is recommended—

(1) That the study of the detection of foreign fats in cacao butter be continued.

(2) That the section, "Examination of fat extracted from milk chocolate", included in the present tentative method for the detection of coconut and palm kernel oils in cacao butter and fat extracted from milk chocolate be changed to read as follows: "Examination of fat extracted from milk chocolate". Milk fat, if present in cacao butter subjected to this test, produces a turbidity less in intensity than that produced by the same percentage of coconut or palm kernel oil. For example, cacao butter containing 10, 15, or 20 per cent milk fat produces, respectively, no opalescence, a faint opalescence, or an opalescence. For this reason, when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply the percentage of lactose in the cacao product by 0.8 or the percentage of casein by 1.1 to obtain the percentage of milk fat in the product, and from this result calculate the percentage of milk fat in the total fat. If this percentage corresponds to 15 per cent or less, a blank of cacao butter containing 15 per cent milk fat may be used, otherwise make up a mixture of cacao butter and milk fat in the proportions indicated by the calculations.

Test the fat extracted from the sample under examination, as directed for the examination of cacao butter, but use the prepared mixture of cacao butter and milk fat instead of the pure cacao butter for the blank. If the fat being tested contains coconut oil or palm kernel oil, the last filtrate, when acidified, will be more turbid or milky than the blank.

REPORT ON NAVAL STORES.

By F. P. VEITCH (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

The work this year was limited to the study of a method for the determination of toluol-insoluble matter in rosin (chiefly sand, chips, dirt and bark).

Three samples of rosin were sent to those who had expressed a willingness to participate in the work. These samples were labeled No. 1, No. 2 and No. 3, and were prepared in the following manner:

Sample No. 1.—A large lump of gum rosin was melted under low heat and poured into a cardboard box to a depth of about 2 inches. When cool the cake of rosin was removed and cut vertically into blocks weighing about 200 grams. Each block was wrapped and numbered.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 13, 78 (1930).

Sample No. 2.—Several lumps of clean gum rosin were melted together with about 10 per cent of sample No. 3. The melted rosin was poured into a cardboard box and stirred as long as possible in an effort to obtain a uniform product. On cooling the rosin cake was cut and wrapped as was No. 1.

Sample No. 3.—Lumps from several barrels of so-called rosin (a product made from cup skimmings) were ground to a powder in a ball mill and thoroughly mixed. This powder was put in envelopes in 75 gram portions, and the envelopes were labeled. On account of high dirt content of this sample it was recommended that only 25 grams be used for a test portion.

The directions sent with the samples were as follows:

(1) If the sample is less than 200 grams, immediately before the determination is made powder it to pass a No. 10 sieve; mix thoroughly; and place in a wide-mouthed bottle of such size that the sample completely fills it.

(2) If the sample is more than 200 grams, crush it to pass a $\frac{1}{4}$ -inch sieve; mix; quarter down to about 200 grams, and treat as described in (1).

PROCEDURE.

Place 50 grams of the freshly-powdered sample in a 300 cc. beaker; add 150 cc. of toluol, free from water and non-volatile residue; and dissolve the sample with the aid of heat and occasional shaking. When solution is apparently complete (no particles of rosin visible), filter at once through a 25 cc. porcelain Gooch crucible which has been previously prepared with a mat of pure well-washed asbestos (such as is used for the determination of barium sulfate) and which has been finally washed thoroughly with the solvent used, dry in a boiling-water oven for 30 minutes, cool in a desiccator, and weigh. If the rosin filtrate is not clear, return it through the Gooch crucible until it is clear, finally washing the residue and the outside of the crucible free from rosin with additional hot solvent. Dry the crucible and contents to constant weight at 105°–110°C. in an oven (1 hour usually suffices), cool in a desiccator, weigh, and calculate the percentage of toluol-insoluble matter.

Four analysts only were able to report results, and to these the referee extends his thanks. Unfortunately but few members of the association are directly interested in this line of work. There is reason to think, however, that this number will increase in the future, especially in the South, which is the rosin-producing section.

The following results, expressed in percentage, were reported:

| | SAMPLE NO. 1 | | SAMPLE NO. 2 | | SAMPLE NO. 3 | |
|--------------------------|--------------|---------|--------------|---------|--------------|---------|
| | | average | | average | | average |
| W. C. Smith | 0.17 | 0.17 | 1.36 | 1.14 | 10.94 | 10.94 |
| Bur. Chemistry and Soils | | | 1.24 | | | |
| | | | 0.82 | | | |
| J. H. Mitchell | 0.16 | 0.16 | 1.26 | 1.23 | 11.02 | 10.95 |
| South Carolina Exp. Sta. | 0.16 | | 1.19 | | 10.88 | |
| A. R. Choppin | 0.12 | 0.14 | 0.14 | 0.16 | 0.19 | 0.18 |
| Louisiana State Univ. | 0.16 | | 0.18 | | 0.17 | |
| G. A. Hawkins and | 0.14 | 0.13 | 0.84 | 0.83 | 12.03 | 11.80 |
| Burton J. Otte | 0.13 | | 0.83 | | 11.44 | |
| University of Florida | 0.13 | | 0.82 | | 11.93 | |

Sample No. 1 represents about the maximum quantity of toluol-insoluble matter which a merchantable rosin should show in samples taken from about 6 inches from the surface of the rosin in the barrel. It is believed, although the data on this point are fragmentary as yet, that sample No. 2 should represent the maximum percentage of toluol-insoluble material of merchantable rosin, as indicated by a sample taken from close to the bottom head. Sample No. 3 was included to represent material containing high percentages of toluol-insoluble constituents. It is evident from the results of Choppin that all the samples sent to him were No. 1 and that he did not have any of sample No. 2 or sample No. 3.

Bearing in mind that this same method has been used cooperatively for two successive seasons by the Naval Stores Committee of the American Society for Testing Materials, the results of which cooperative work are in substantial agreement with those reported here, the referee feels that the method is quite satisfactory and has been proved out sufficiently to warrant recommendation for adoption as a tentative method.

It is recommended¹, therefore, that the method presented for the determination of toluol-insoluble matter in rosin (chiefly sand, chips, dirt and bark) be adopted as a tentative method of the association.

No report on turpentine was given by the associate referee.

No report on paints, paint materials and varnishes was given by the referee.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 13, 63 (1930).

CONTRIBUTED PAPERS.

THE IDENTIFICATION OF ALKALOIDS BY PRECIPITATION.

I. A NATURAL CLASSIFICATION OF THE ALKALOIDS BASED ON PRECIPITATION.

By CHARLES C. FULTON (U. S. Industrial Alcohol Bureau, Omaha, Neb.).

One of the most important characteristics of any alkaloid is the relative sensitivity toward it of the different precipitating agents. The examination of dilute alkaloidal solutions provides a natural classification of the alkaloids, and even provides a means of tentatively identifying any alkaloid by precipitation, without the use of the microscope. When it is fully developed and combined with microscopic tests, the method makes possible the identification of any known and studied pure alkaloid, and not more than 5 mg. is ever required for the tests.

In obtaining the relative sensitivities it is most convenient to compare all the other reagents to phosphomolybdic acid, which makes the best standard. The method is as follows:

Use a solution of the alkaloidal salt in water, or the alkaloid dissolved in dilute acid, preferably sulfuric. Test one drop of this solution on the microscope slide with a drop of 10 per cent phosphomolybdic acid. If a heavy precipitate is obtained, dilute a portion of the alkaloidal solution with an equal volume of water. Repeat this dilution until a solution is obtained that will just give a distinct and unmistakable precipitate with phosphomolybdic acid, but no more. Call this "solution 1", as containing the unit quantity of alkaloid. Solution 1 is nearly always considerably weaker than 1 to 1000. The next stronger solution, containing twice as much alkaloid per unit volume, is called solution 2; a solution containing half as much alkaloid is called solution $\frac{1}{2}$, and so on. In this way the sensitivity of any reagent relative to phosphomolybdic acid can be specified. The system of classification and tentative identification requires tests only on solution 1, but tests on stronger and weaker solutions are helpful. Make tests on solution 1 with the various reagents, using a drop of solution and a drop of reagent on a glass slide. These tests show in each case whether the reagent is at least as sensitive as phosphomolybdic acid to the particular alkaloid, or less so.

RESULTS.

When several different alkaloids have been tested by the foregoing method, it will be found that the reagents can be so arranged that the effect of many reagents can be predicted with certainty from the result obtained with the first reagent tried. If, for instance, picric acid precipitates the alkaloid in solution 1, then Mayer's reagent, gold chloride, and bromine water will also precipitate it; while if picric acid fails to precipitate solution 1, then platinum chloride, potassium chromate, and potassium hydroxide will also fail. The alkaloids can be similarly arranged.

If alkaloids and reagents are arranged in columns, one ascending and one descending, they can be placed opposite each other in such a way as to show that an alkaloid is precipitated in solution 1 by all the reagents standing above it, and by none of those below it. The following is such an arrangement for some of the principal alkaloids and reagents:

| | |
|---------------------------|----------------------------|
| Phosphomolybdic acid..... | Caffeine |
| Wagner's reagent..... | Atropine |
| Gold chloride..... | Morphine |
| Marme's reagent..... | Cocaine |
| Picric acid..... | Diacetylmorphine (Heroine) |
| Ammonium molybdate..... | Quinine |
| Platinum chloride..... | Strychnine |
| Potassium chromate..... | Papaverine |

If an attempt is made to extend this scheme to all the alkaloids and reagents, certain inconsistencies develop. These inconsistencies, with the exception of a few due to oxidation, disappear if the alkaloids are arranged in groups. The classification so obtained is an entirely natural one.

CLASSIFICATION.

In this classification by precipitation it is not possible to separate the alkaloids from other basic compounds precipitated by the same reagents. They should be considered together. In the following table, which shows how the scheme is extended, the alkaloids and amines are separated into groups. This table includes most of the alkaloids that the writer has studied thus far, but it omits many reagents. It will be necessary to specify exactly the formulas for the various reagents before all of them can be put in their proper places, and in some cases it will be necessary to change the usual formulas.

| |
|---------------------------|
| (1) |
| Phosphotungstic acid..... |
| Phosphomolybdic acid..... |
| <i>Ammonia</i> |

| |
|----------------------------|
| (3) |
| Bromine water..... |
| Wagner's reagent..... |
| (saturated with iodine) |
| Dragendorff's reagent..... |
| <i>Aniline</i> |
| <i>Pyridine</i> |

| |
|--------------------|
| (2) |
| Bromine in HBr |
| Acid Wagner's |
| <i>Ecgonine</i> |
| Acid Dragendorff's |
| <i>Caffeine</i> |

| | |
|------------------------------|------------------|
| (4) | |
| Wagner's reagent..... | |
| (Stephenson's formula) | |
| Mayer's reagent..... | |
| | <i>Atropine</i> |
| | <i>Morphine</i> |
| Mercuric sodium bromide..... | |
| | <i>Codeine</i> |
| | <i>Novocaine</i> |

| | |
|-------------------------|------------------|
| (5) | |
| Marme's reagent..... | |
| | <i>Cocaine</i> |
| | <i>Colarnine</i> |
| Picric acid..... | |
| | <i>Heroine</i> |
| Ammonium molybdate..... | |
| | <i>Narceine</i> |

| | |
|-------------------------|---------------------|
| (6) | |
| Palladium chloride..... | |
| | <i>Cinchonidine</i> |
| | <i>Quinine</i> |
| Platinum chloride..... | |
| | <i>Brucine</i> |
| | <i>Strychnine</i> |
| Chromic anhydride..... | |

| | |
|-------|------------------------|
| (7) | |
| | Potassium chromate |
| | <i>Apomorphine</i> |
| | Concentrated Potassium |
| | Acetate |
| | <i>Pseudomorphine</i> |
| | Potassium hydroxide |
| | <i>Narcotine</i> |
| | Palladium chloride |
| | Platinum chloride |
| | Chromic anhydride |
| | <i>Papaverine</i> |

| | |
|--------------------------------------|-------------------------------|
| (8) | |
| Hydroferricyanic acid..... | |
| SnCl ₄ and Conc. HCl..... | |
| | <i>Protein of blood serum</i> |

| | |
|-------|-------------------------|
| (9) | |
| | Potassium ferricyanide |
| | Saccharin |
| | <i>Fuchsine (basic)</i> |
| | <i>Malachite green</i> |

There are six classes of alkaloids, and many (probably most) of the amines will fall in the same classes. One other class is needed for basic substances simpler than any of the alkaloids and at least two other classes for substances more complex and more easily precipitated, such as proteins and basic dyes. The writer believes that the six classes, 2 to 7, inclusive, as described here, will take in all the alkaloids that have been obtained in pure form and thoroughly studied up to the present time.

An outline like the one just given, showing the relations of alkaloids and reagents, has many uses. For instance, a reagent that shows but little sensitivity to papaverine and strychnine will certainly not be sensitive to any of the other alkaloids. Again, it is usually possible to tell from the table whether a certain alkaloid can be detected by precipitation in the presence of another alkaloid, or whether the two can be separated by precipitation; and, further, it can be seen with which reagents this may be possible and with which it is clearly impossible.

The different classes will now be described separately, and the writer

will show that the classification corresponds to the data given by Stephenson¹ for precipitation from 2 per cent solutions, and that it brings together alkaloids that are related in certain ways, particularly in respect to solubility and basic strength.

| GENERAL CHARACTERISTICS OF THE CLASS | CHARACTERIZATION BY PRECIPITATION | STEPHENSON'S DATA— NUMBER OF PRECIPITATES OBTAINED | |
|---|-----------------------------------|---|----------------------------|
| | | With 7 basic reagents | With all 35 reagents |

(1) Potassium—Ammonia class.

Examples: potassium, ammonia.

| | | |
|---|---|---------------|
| The simplest substances that can be precipitated by the alkaloidal reagents: the heavier alkali metals, ammonia, and the simplest aliphatic amines. Strong bases, easily soluble in water. Some, or perhaps all, of the nitrogenous bases of the class are gases. | These bases are precipitated by phosphomolybdic acid; phosphotungstic acid is more sensitive. They are also precipitated by picric acid, chloroplatinic acid, and a few other reagents, though these are usually not very sensitive. Bromines in HBr, Wagner's, Mayer's, and the majority of the alkaloidal reagents ordinarily do not even precipitate these substances. | (None tested) |
|---|---|---------------|

REMARKS.—The class has two subgroups: the heavier alkali metals and the nitrogenous bases. They react similarly in acid solution, but tests on solutions of the free bases readily distinguish them, e. g., ammonium, but not potassium, hydroxide solution gives a precipitate with Wagner's reagent.

(2) Caffeine class.

Examples: caffeine, ecgonine.

| | | |
|---|---|---------------|
| The simplest, weakest basic substances that can be called alkaloids. They are generally freely soluble in water and alkali. | Bases in this and in all subsequent classes are at least moderately sensitive to phosphomolybdic and phosphotungstic acids and to bromine in HBr. Those of this class are not sensitive to neutral reagents and usually are not even precipitated. In a strongly acidified test drop they are fairly sensitive to Wagner's and Dragendorff's reagents, but usually even then lack sensitivity to Mayer's. Excess KI markedly reduces the sensitivity of these reagents. Chlorauric acid precipitates these bases, but it is not sensitive. The basic reagents and most of the minor reagents fail to precipitate. | (Theobromine) |
| | | 0 5 |
| | | Ecgonine |
| | | 0 7 |
| | | Caffeine |
| | | 0 8 |
| | | (Colchicine) |
| | | 0 14 |

REMARKS.—In the last column, parentheses are used to enclose the names of alkaloids not studied by the writer, but which, judging from Stephenson's data and other sources, apparently belong to the class. The alkaloids and amines listed as "examples" are those studied by the writer.

¹ Some Microchemical Tests for Alkaloids. J. B. Lippincott, 1921.

(3) Aniline—Pyridine class.

Examples: aniline, pyridine, alpha and beta naphthalamine, hexamethylene-tetramine.

The simplest aromatic amines and similar substances make up this class. They are fairly strong bases, usually moderately soluble in water. Many of them are liquids.

Bases of this class are sensitive to bromine water and to Wagner's reagent, if saturated with iodine. Very little excess KI suffices to destroy the sensitivity to Wagner's; none of these bases are precipitated from solution 1 by Stephenson's form of the reagent. They usually lack sensitivity to Mayer's reagent but are fairly sensitive to Dragendorff's. They do not give a precipitate in solution 1 with chlorauric acid unless it oxidizes the base, but this often occurs. They lack sensitivity to most of the alkaloidal reagents. The free bases, unlike those of the caffeine class, are precipitated by Wagner's and Mayer's reagents, but the crystals and sensitivities are not the same as with acid solutions.

(Arecoline)
0 14

(Coniine)
0 16

(Cytisine)
0 16

(Pilocarpine)
0 16

REMARKS.—The writer has not been able to study any alkaloids belonging to this class, but there seems little doubt but that arecoline, coniine, cytisine, and pilocarpine do belong to it.

Alkaloids of this and of the caffeine class often give crystalline precipitates with the complex oxygen acids, while those of subsequent classes practically always give amorphous precipitates. All of the ten crystalline precipitates obtained by Stephenson with phosphotungstic and silicotungstic acids, and six out of nine of those obtained with phosphomolybdic acid, were given by alkaloids of this or of the caffeine class.

(4) Atropine class.

Examples: atropine, homatropine, codeine, morphine, novocaine.

Strong bases, moderately to slightly soluble in water. (2% or more of codeine will dissolve, but less than $\frac{1}{2}$ of 1% of morphine.)

All alkaloids of this and subsequent classes are precipitated in solution 1 or even more dilute solution by bromine water, Wagner's, Dragendorff's, and Mayer's reagents. The sensitivity of reagents containing KI is noticeably reduced by an excess, but this phenomenon is much less marked than with the bases of the aniline-pyridine class. These bases are not precipitated in solution 1 by Marne's reagent, picric acid, or ammonium molybdate; this is true also of all the bases of the preceding classes, with the exception of a possible picric acid precipitate with a few of the bases of class (1), where the sensitivity to phosphomolybdic acid is quite low. In this class solutions of the free bases give about the same results as acidified solutions.

Atropine
2 17

Homatropine
1 18

(Hyoascyamine)
3 21

(Physostigmine)
2 18

Codeine
1 20

Morphine
2 22

REMARKS.—This and the next two classes contain the most typical alkaloids.

(5) Cocaine—Cotarnine class.

Examples: (a) cocaine, heroine, narceine; (b) cotarnine.

This class is not easy to define as a whole. It contains: (a) insoluble bases of somewhat variable strength; (b) certain soluble bases.

The two groups of alkaloids constituting this class are not distinguishable by sensitivity in solution 1, but can be separated by basic reagents and in other ways in more concentrated solutions. They are all precipitated in solution 1 by gold chloride and by Marme's reagent. Their sensitivity to reagents containing KI is practically unaffected by an excess of KI; this is also true of bases of subsequent classes. Bases of this and of the preceding classes are not precipitated in solution 1 by palladium chloride or K_2CrO_4 .

| | |
|--------------|----|
| (a) | |
| Cocaine | |
| 3 | 24 |
| Heroine | |
| 4 | 26 |
| Narceine | |
| 2 | 27 |
| (b) | |
| Hydrastinine | |
| 1 | 20 |
| (Berberine) | |
| 1 | 28 |

REMARKS.—The only basic reagent precipitating cotarnine or hydrastinine is KCN. This precipitation appears to be due to the fact that these alkaloids are aldehydes. (Berberine also is precipitated only by KCN.)

(6) Quinine class.

Examples: cinchonidine, quinine, quinidine, brucine, strychnine.

Very insoluble strong bases

These bases are very insoluble, but so sensitive to phosphomolybdic acid that as a rule they are not precipitated from solution 1 by any basic reagent. They are all precipitated from solution 1 by picric acid and by ammonium molybdate, and they are also sensitive to palladium chloride.

| | |
|--------------|----|
| (Cinchonine) | |
| 5 | 29 |
| Cinchonidine | |
| 6 | 33 |
| Quinidine | |
| 5 | 32 |
| Quinine | |
| 7 | 33 |
| (Thebaine) | |
| 5 | 30 |
| Brucine | |
| 4 | 29 |
| Strychnine | |
| 5 | 32 |

REMARKS.—As these are strong bases, they are not, as a rule, precipitated by potassium acetate, and often not by sodium phosphate or benzoate. The precipitation of quinine by all the basic reagents is due to the fact that it is a di-acid base; the neutral salts are much less soluble than the acid salts, and these, not the free alkaloid, are precipitated in several cases.

Most alkaloids forming insoluble salts with the ordinary laboratory acids, and also those precipitated from alcohol by iodine, belong to this or the following class.

(7) Narcotine class.

Examples: apomorphine, pseudomorphine, narcotine, papaverine.

Very insoluble weak bases

These bases are precipitated by K_2CrO_4 from solution 1. They are precipitated by weak basic reagents. Several are phenols, and so may fail of precipitation by strong alkali, or be redissolved, at least in dilute solution. As a rule they are not precipitated in solution 1 by palladium chloride; but like the bases of the preceding class they are precipitated in solution 1 by picric acid and by ammonium molybdate.

| | |
|--------------|----|
| (Apocodeine) | |
| 5 | 33 |
| Apomorphine | |
| 7 | 35 |
| (Hydrastine) | |
| 7 | 34 |
| Narcotine | |
| 7 | 34 |
| Papaverine | |
| 7 | 35 |

REMARKS.—Apomorphine and papaverine were the only alkaloids tested by Stephenson that gave precipitates with all the 35 reagents used.

(8) Protein class.

Example: the protein of blood serum.

If the test drop is slightly acid, such bases are sensitive to all the reagents to which any of the alkaloids are sensitive, with the exception of the basic reagents. In addition, they are precipitated in solution 1 by such reagents as chromic anhydride and mercuric nitrate; by ammonium thiocyanate, potassium ferricyanide, and sodium nitroprusside, if the test drop is acid; and by zinc chloride, or stannous chloride in concentrated hydrochloric acid.

(9) Basic dye class.

Examples: fuchsin (basic), malachite green.

Fuchsin and malachite green, representatives of the basic dyes, are sensitive to the reagents showing sensitivity to alkaloids, including basic reagents. In addition they are sensitive to such reagents as chromic anhydride, potassium ferricyanide, sodium nitroprusside, zinc chloride, saccharin, etc.

SUMMARY.

1. A natural classification of the alkaloids based solely on precipitation from dilute solutions is possible. Amines and similar basic substances precipitated by the same reagents are included in the scheme.

2. The key to the classification is the relative sensitivity of the different reagents to each alkaloid. For convenience, all the other reagents are compared with phosphomolybdic acid.

3. The classification so obtained corresponds in certain ways to the data of precipitation from 2 per cent solutions, and to the solubility in water and the basic strength of the free alkaloids.

4. The alkaloidal solutions required are practically never more concentrated than 1 to 1000. Hence, a few milligrams of an alkaloid suffice for its classification and even for the tentative identification of an unknown. Fully developed and combined with microscopic tests, the system will ensure the identification of any pure alkaloid that has been studied, and not more than 5 mg. will ever be required.

THE QUANTITATIVE DETERMINATION OF RADIUM BY THE EMANATION METHOD.

By RALPH G. FULTON (University of Missouri, Columbia, Mo.).

At the request of J. W. Sale, Referee on Radioactivity in Drugs and Water, collaborative work in testing out the tentative methods for radium analyses was undertaken at the University of Missouri. The methods submitted are modifications of the Boltwood method; they are given in detail in Sale's report¹. A water sample, a water-soluble salt and a refractory substance requiring sodium carbonate fusion and hydrofluoric acid treatment were used as samples.

¹ *This Journal*, 8, 531 (1925).

In brief, the procedure consists in dissolving a definite weight of the samples, boiling the solution to remove all the accumulated emanation, sealing, and allowing the emanation to accumulate for a stated period of time. The accumulated emanation is then separated from the solution by boiling and collected in a suitable buret. The emanation, or radon, is then transferred to a calibrated electroscope, where the amount is determined by measuring the drift of the charged leaf system.

APPARATUS.

The apparatus selected for this work consisted of four ionization chambers, three of which had a volume of about 4 liters, and the fourth, a volume of about 10 liters. These chambers were used in connection with two Lind¹ interchangeable-type electroscope heads, one having a gold leaf, and the other, which was slightly less sensitive, an aluminum leaf.

Inasmuch as no standard radium solution or mineral of known radium content was supplied, a standard solution prepared by H. Barker² and a sample of analyzed pitchblende, the radium content of which had been determined by M. Randall³, were used as two independent standards. The results obtained on both standards were in good agreement, ranging around 10^{-8} curies of radon per net drift of one division per second of the electroscope leaf. The range is illustrated in a typical case in which the value 1.378×10^{-8} represents the average standardization factor from six trials, the individual values ranging between 1.36×10^{-8} and 1.44×10^{-8} curies per division per second.

The instructions for setting up were followed carefully, and the apparatus proved quite satisfactory. Trouble was encountered, however, when the rubber stopper of the gas collecting buret came out while the buret was being filled with the hot lye solution. In order to avoid this trouble, glass burets of one piece were substituted for both gas collecting and transfer burets; besides preventing leakage, they simplified the gas transfer operation by having two-way stopcocks at the upper ends. The modified form is shown in Figure 1. Another change was made for the sake of convenience—the insertion of the two-way stopcock (10), which made it possible to fill the reservoir (9) of the transfer assembly from the lye boiler (2), by means of the tap (12). In order to make certain that no water vapor or calcium chloride dust would be carried into the ionization chamber, a separate cotton filter tube (11) was added, so that the drying tube (7) could be completely filled with calcium chloride. None of these changes affected the results, and the insertion of the all glass burets did much to simplify the procedure.

¹ U. S. Bur. Mines Bull. 104, p. 99.

² Univ. Mo. Bull., Vol. 24, No. 26, p. 54.

³ Univ. Mo. thesis, 1926.

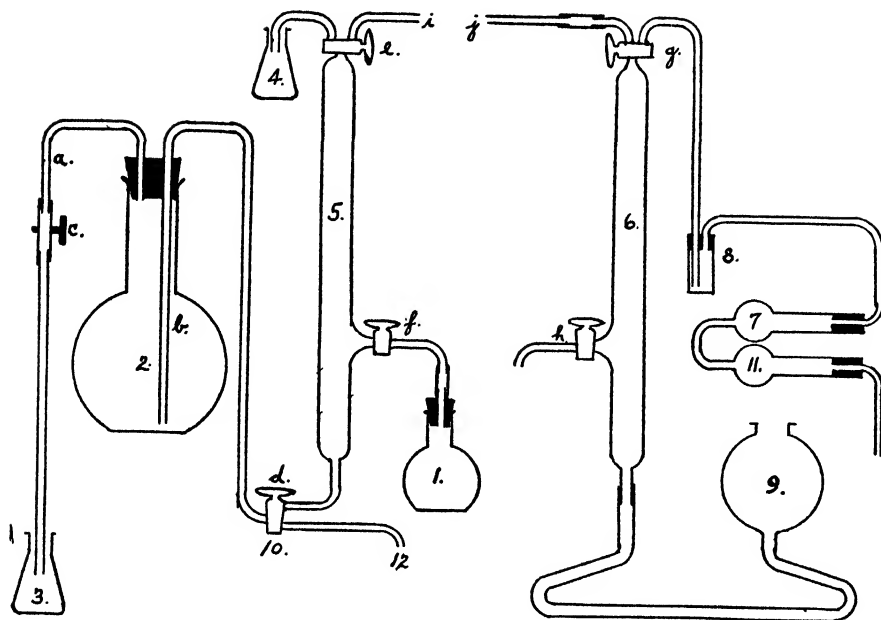


FIGURE 1.

The results obtained and a comparison with the actual radium content of the samples are given in Table 1.

TABLE 1.

| | A. O. A. C. SAMPLE NO. 1 (WATER) 10 ⁻³ g. radium per 100 cc. | A. O. A. C. SAMPLE NO. 2 (SALT) 10 ⁻³ g. radium per 5 grams | A. O. A. C. SAMPLE NO. 3 (REFRACTORY) 10 ⁻³ g. radium per 250 mg. |
|-----------------------|---|--|--|
| Found. | 3.16 | 3.13 | 3.97 |
| Found..... | 3.19 | 3.16 | 4.11 |
| Found..... | 3.16 | 3.01 | 3.95 |
| Average | 3.17 | 3.10 | 4.01 |
| Present..... | 3.05 | 3.06 | 4.08 |
| Error (per cent)..... | +3.94 | +1.31 | -1.71 |

In order to ascertain whether or not the methods for the determination of radium in use at the University of Missouri would give concurring values for these samples, the Schlundt and Moore¹ apparatus, shown in Figure 2, was used as a check method. The procedure, which is very simple, is equivalent to boiling off the emanation directly into the transfer buret of the apparatus shown in Figure 1. The results obtained by this method, expressed in the same units as given in Table 1, were also in good agreement with the true values, as shown in Table 2.

¹ *J. Phys. Chem.*, 9, 320 (1905).

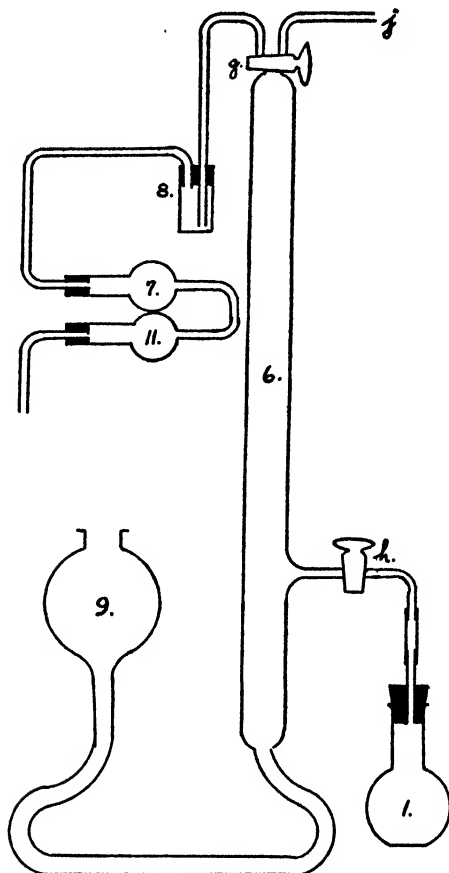


FIGURE 2.

TABLE 2.

| | A. O. A. C. METHOD NO. 1 | A. O. A. C. METHOD NO. 2 | A. O. A. C. METHOD NO. 3 |
|-----------------------|-----------------------------|-----------------------------|-----------------------------|
| Average value..... | 3.12 | 3.03 | 4.084 |
| Error (per cent)..... | -2.3 | -1.6 | +0.09 |

A third check method, the bisulfate fusion method developed by Barker¹, was used for the refractory substance, Sample No. 3. The apparatus for this determination is shown in Figure 3. (A) and (D) are micro drying bulbs containing concentrated sulfuric acid; (B) is the storage container, an $8 \times \frac{3}{4}$ inch Pyrex test tube; (C) is a similar test tube containing stick sodium hydroxide, and (E) is a cotton filter tube. In brief, the procedure is as follows: A weighed portion of the sample is mixed with sufficient fused potassium bisulfate to fill the test tube about one-third full after fusion, a little barium carbonate is added, and

¹ *J. Ind. Eng. Chem.*, 30, 525 (1918).

the mixture is placed in the Pyrex test tube and boiled for at least 5 minutes. After the melt has cooled somewhat, a two-hole rubber stopper fitted with glass tubing, the outer ends of which have been drawn out to sealed capillaries, is inserted, and the time of sealing is noted. After a period of storage, the emanation that has accumulated is removed, and its quantity is determined by the effect on the rate of discharge of a charged electroscope. For this purpose the storage container is connected in the train as shown in the figure, a good grade of tight-fitting rubber tubing being used. The evacuated ionization chamber is connected to (E). The capillary tips are broken by pinching the surrounding rubber tubing with pliers, the time is noted, and the stopcock of the evacuated ionization chamber is partially opened so that a slow, steady current of air is drawn through the train. The storage flask is heated until the contents of the tube have boiled for several minutes, the air current sweeping all the emanation into the electroscope chamber. The purpose of the sodium hydroxide tube (C) is to remove the oxides of sulfur and prevent their introduction into the chamber. A strong solution of sodium hydroxide or potassium hydroxide in a micro drying bulb is often used in place of the tube (C). The cotton tube (E) prevents sulfuric acid spray from being drawn into the chamber. From the rate of drift, the standardization factor of the instrument and the period of storage, the radium content of the sample may be calculated. The average value obtained for Sample No. 3 by this method was 4.04 millimicrograms per 250 mg. of sample, which compares quite favorably with the true value of 4.08 millimicrograms.

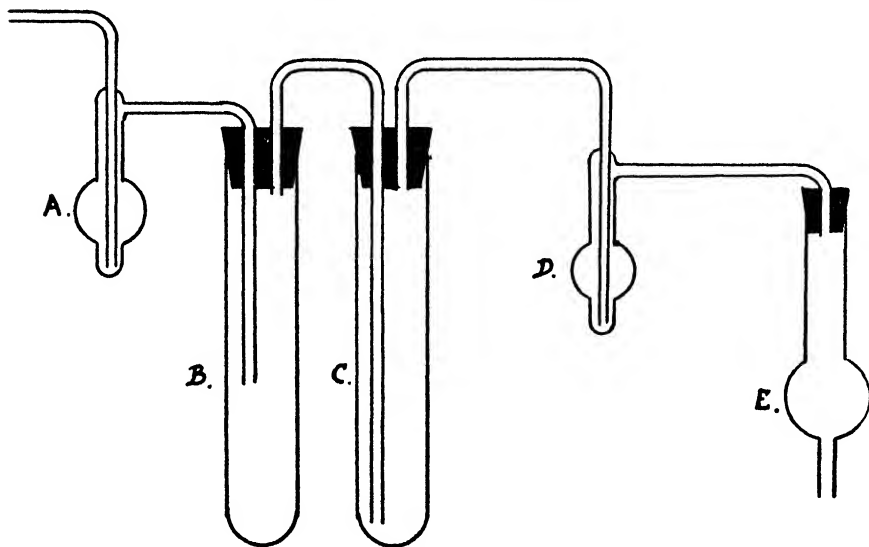


FIGURE 3.

Upon inquiry, it was learned that the refractory substance, Sample No. 3, consisted of a radium salt mixed with kaolin. The possibility of the kaolin alone being the refractory part of the sample then suggested itself. To determine whether or not this was the case, 250 mg. of the substance was made up into a sample according to the instructions for the soluble salt sample, No. 2. The resulting solution, of course, contained a precipitate of kaolin. This sample was sealed, stored and tested, and the result was in good agreement with the true radium content, showing that for this particular sample the fusions and hydrofluoric acid treatments were unnecessary. Sample No. 3, therefore, did not appear to be a typical refractory radioactive material.

CYRTOLITE ORE TEST.

Having had occasion to test a refractory cyrtolite ore, containing zirconium, hafnium and uranium, for its radium content, the writer considered it pertinent to assay this ore by the procedure given for the refractory substance. A 500 mg. sample, ground to pass an 80-mesh sieve, was taken. Instructions were followed through two sodium carbonate fusions and hydrofluoric acid treatments, but there always remained a gray residue that apparently was not affected by such treatment. The clear solution of the combined filtrates up to this point was sealed as one sample, Sample No. A, and the residue was placed in a nickel crucible and fused with sodium peroxide. The melt was dissolved and acidified with nitric acid, and the clear solution obtained was likewise sealed up as a sample, Sample No. B. After storage, the two samples were tested for their activity; it was found that all the activity was in Sample A and that the radium content agreed with values obtained by putting a sample completely into solution by introducing a sodium peroxide fusion into the procedure (Sample No. C). Apparently this indicates that it is possible to have all the radioactive material in solution, although a considerable amount of the refractory material is not in solution, which bears out the results obtained by treating the A. O. A. C. No. 3 refractory sample as a soluble material. Whether this can be applied to all refractory radioactive materials is quite doubtful.

This same cyrtolite ore was also tested by the bisulfate fusion method, but the results were low. The values obtained were as follows:

| | URANIUM per cent |
|-------------------------------------|---------------------|
| *Sample A (combined filtrates)..... | 6.14 |
| Sample B (peroxide fusion)..... | nil |
| *Sample C (complete solution)..... | 6.20 |
| Sample D (bisulfate fusion)..... | 4.24 |
| Sample E (bisulfate fusion)..... | 5.00 |

* The per cent uranium determined analytically was in good agreement with these values.

SUMMARY.

The results of this cooperative experiment may be summarized as follows:

(1) The methods proposed by Sale (tentative methods) for examining radioactive material were applied to (a) a water sample, (b) a soluble salt sample and (c) a refractory substance and found to be reliable.

(2) Several modifications resulting in simplicity and convenience, without loss of accuracy, are suggested.

(3) The tentative method was checked by: (a) the Schlundt and Moore method, and (b) the bisulfate fusion method developed by Barker, both methods in general use at the University of Missouri in making radium assays.

(4) The procedure was extended to include more refractory substances than that submitted, and it was found to give accurate results.

These experiments were conducted in the chemical laboratory of the University of Missouri. Dr. Herman Schlundt gave helpful suggestions.

THE CHEMICAL COMPOSITION OF AUTHENTIC SAMPLES OF WHOLE WHEAT FLOURS AND MODIFIED WHOLE WHEAT FLOURS*.

By L. H. BAILEY (Bureau of Chemistry and Soils) and S. C. ROWE (Food and Drug Administration, U. S. Department of Agriculture).

It is well known that wheats of different classes and wheats of the same class grown under different soil and climatic conditions vary considerably in their chemical composition. It is also known that the so-called whole wheat, entire wheat or graham flours on the market today vary widely in their composition owing to the different processes of manufacture used. This investigation was undertaken with a view to obtaining data which might be of value in differentiating between the flour obtained by simply grinding the wheat berry, with nothing added and nothing taken away, and those flours in which the bran content is modified.

Six samples of wheat consisting of hard red spring, durum, hard red winter, white and two soft red winter wheats from the eastern and central sections of the country were selected for the work. This material was carefully milled in the laboratory¹. Three divisions of each sample were then prepared, making a total of eighteen samples for analysis. The divisions were as follows: (a) the ground wheat berry; (b) the ground wheat berry sifted to effect a 10 per cent by weight bran removal; (c) the ground wheat berry plus bran to the extent of 10 per cent of the weight of the wheat.

* Contribution No. 94 from Food Research Division.

¹ Acknowledgment is made to the Milling Division of the Bureau of Agricultural Economics for their cooperation in milling the samples.

TABLE

Chemical composition of whole wheat and modified

| NO. | KIND | MOIS- TURE | PRO- TEIN (N × 5.7) | LIPIDS | SUGARS | STARCH |
|-----|--|-----------------|------------------------------|-----------------|-----------------|-----------------|
| | | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1 | Hard red spring wheat flour | 12.66 | 12.99 | 2.87 | 2.98 | 57.73 |
| 2 | " " " " " -10% bran | 12.45 | 12.85 | 2.82 | 2.69 | 60.84 |
| 3 | " " " " " +10% bran | 12.22 | 13.22 | 3.06 | 3.33 | 52.53 |
| 4 | Durum wheat flour | 11.80 | 13.65 | 3.22 | 2.90 | 55.54 |
| 5 | " " " " " -10% bran | 11.80 | 13.82 | 2.96 | 2.63 | 61.89 |
| 6 | " " " " " +10% bran | 11.65 | 13.68 | 3.23 | 3.06 | 52.83 |
| 7 | Hard red winter wheat flour | 11.85 | 12.33 | 2.51 | 2.38 | 58.89 |
| 8 | " " " " " -10% bran | 11.78 | 11.97 | 2.46 | 2.08 | 63.68 |
| 9 | " " " " " +10% bran | 12.32 | 12.42 | 2.54 | 2.54 | 54.95 |
| 10 | Soft red winter wheat flour (St. Louis) | 12.49 | 10.60 | 2.36 | 2.05 | 60.07 |
| 11 | " " " " " -10% bran | 12.32 | 10.55 | 2.35 | 1.79 | 66.23 |
| 12 | " " " " " +10% bran | 12.12 | 10.91 | 2.37 | 2.17 | 57.39 |
| 13 | White wheat flour | 11.62 | 13.93 | 2.73 | 3.27 | 57.07 |
| 14 | " " " " " -10% bran | 11.57 | 13.62 | 2.46 | 3.15 | 61.19 |
| 15 | " " " " " +10% bran | 11.62 | 13.93 | 2.63 | 3.55 | 53.81 |
| 16 | Soft red winter wheat flour (Baltimore) | 11.99 | 9.58 | 2.89 | 2.60 | 61.02 |
| 17 | " " " " " -10% bran | 12.45 | 9.20 | 2.76 | 2.32 | 64.31 |
| 18 | " " " " " +10% bran | 12.13 | 9.66 | 2.92 | 2.80 | 57.94 |
| | Whole wheat flour: { Maximum | 12.66 | 13.93 | 3.22 | 3.37 | 61.02 |
| | { Minimum | 11.62 | 9.58 | 2.36 | 2.05 | 55.54 |
| | { Average | 12.07 | 12.18 | 2.76 | 2.71 | 58.39 |
| | Whole wheat flour minus 10% as bran: { Maximum | 12.45 | 13.82 | 2.82 | 3.15 | 66.23 |
| | { Minimum | 11.57 | 9.20 | 2.35 | 1.79 | 60.84 |
| | { Average | 12.06 | 12.00 | 2.64 | 2.44 | 63.02 |
| | Whole wheat flour plus 10% as bran: { Maximum | 12.32 | 13.93 | 3.23 | 3.55 | 57.94 |
| | { Minimum | 11.62 | 9.66 | 2.37 | 2.17 | 52.53 |
| | { Average | 12.01 | 12.30 | 2.79 | 2.91 | 54.91 |

* The analyses were made by the official and tentative methods of the A. O. A. C.—
 Moisture by the air-oven method, 130°C. for 1 hr., *This Journal*, 9, 40 (1926).
 Starch by the direct method, *Ibid.*, 11, 37 (1928).
 Ash by the glycerine-alcohol method, *Ibid.*, 8, 671 (1925).
 The other methods are given in *Methods of Analysis*, A. O. A. C., 1925.

These eighteen samples were analyzed for moisture, protein, lipids, sugars, starch, ash, calcium oxide, magnesium oxide, crude fiber and pentosans. From the results obtained ratios were calculated between calcium oxide and magnesium oxide, starch and lipids, starch and crude fiber, starch and ash, starch and pentosans, and pentosans and ash. The results of these analyses and calculations are presented in the table. Only average results are given, these having been obtained from closely agreeing duplicates and in the case of starch from triplicates. The

1.

whole wheat flours—authentic samples.*

| ASH | CaO | MgO | CRUDE FIBER | PENTOSANS | CaO X 100 | STARCH | STARCH | STARCH | STARCH | PROTEIN | TOTAL |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------|---------|-------------|--------|-----------|---------|-----------------|
| | | | | | MgO | LIPOIDS | CRUDE FIBER | ASH | PENTOSANS | ASH | |
| <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | | | | | | | <i>per cent</i> |
| 1.608 | 0.052 | 0.246 | 2.56 | 6.99 | 21.1 | 20.1 | 22.5 | 35.9 | 8.3 | 8.1 | 100.39 |
| 1.197 | 0.044 | 0.172 | 1.48 | 5.24 | 25.6 | 21.6 | 41.1 | 50.8 | 11.6 | 10.7 | 99.57 |
| 2.053 | 0.061 | 0.323 | 3.59 | 8.78 | 18.9 | 17.2 | 14.6 | 25.6 | 6.0 | 6.4 | 98.78 |
| 1.549 | 0.055 | 0.227 | 2.38 | 6.36 | 24.2 | 17.2 | 23.3 | 35.9 | 8.7 | 8.8 | 97.40 |
| 1.243 | 0.046 | 0.180 | 1.21 | 4.77 | 25.6 | 20.9 | 51.1 | 49.8 | 13.0 | 11.1 | 100.12 |
| 1.806 | 0.062 | 0.265 | 3.28 | 7.90 | 23.4 | 16.4 | 16.1 | 29.3 | 6.7 | 7.6 | 97.44 |
| 1.745 | 0.066 | 0.249 | 2.23 | 6.96 | 26.5 | 23.5 | 26.4 | 33.7 | 8.5 | 7.1 | 98.90 |
| 1.279 | 0.057 | 0.169 | 1.36 | 5.45 | 33.7 | 25.9 | 46.8 | 49.8 | 11.7 | 9.4 | 100.06 |
| 2.161 | 0.074 | 0.309 | 3.15 | 8.52 | 23.9 | 21.6 | 17.4 | 25.4 | 6.4 | 5.7 | 98.61 |
| 1.791 | 0.061 | 0.225 | 2.41 | 7.46 | 27.1 | 25.5 | 24.9 | 33.5 | 8.1 | 5.9 | 99.23 |
| 1.210 | 0.052 | 0.154 | 1.37 | 5.36 | 33.8 | 28.2 | 48.3 | 54.7 | 12.4 | 8.7 | 101.18 |
| 2.213 | 0.074 | 0.305 | 3.21 | 8.86 | 24.3 | 24.2 | 17.9 | 25.9 | 6.5 | 4.9 | 99.24 |
| 1.646 | 0.056 | 0.238 | 2.50 | 6.92 | 23.5 | 20.9 | 22.8 | 34.7 | 8.2 | 8.5 | 99.79 |
| 1.269 | 0.046 | 0.166 | 1.62 | 5.70 | 27.7 | 24.9 | 37.8 | 48.2 | 10.7 | 10.7 | 100.58 |
| 2.000 | 0.062 | 0.288 | 3.18 | 8.40 | 21.5 | 20.5 | 16.9 | 26.9 | 6.4 | 7.0 | 99.12 |
| 1.716 | 0.051 | 0.231 | 2.41 | 6.91 | 22.1 | 21.1 | 25.3 | 25.6 | 8.8 | 5.6 | 99.12 |
| 1.210 | 0.043 | 0.166 | 1.58 | 5.42 | 25.9 | 23.3 | 40.7 | 53.1 | 11.9 | 7.6 | 99.25 |
| 2.107 | 0.057 | 0.283 | 3.19 | 7.68 | 20.1 | 19.8 | 18.2 | 27.5 | 7.5 | 4.6 | 98.43 |
| 1.79 | 0.066 | 0.249 | 2.56 | 7.46 | 27.1 | 25.5 | 26.4 | 35.9 | 8.8 | 8.8 | |
| 1.55 | 0.051 | 0.225 | 2.23 | 6.36 | 21.1 | 17.2 | 22.5 | 33.5 | 8.1 | 5.6 | |
| 1.68 | 0.057 | 0.236 | 2.42 | 6.93 | 24.1 | 21.4 | 24.2 | 34.9 | 8.4 | 7.3 | |
| 1.28 | 0.057 | 0.180 | 1.62 | 5.70 | 33.8 | 28.2 | 51.1 | 54.7 | 13.0 | 11.1 | |
| 1.20 | 0.043 | 0.154 | 1.21 | 4.77 | 25.6 | 20.9 | 37.8 | 48.2 | 10.7 | 7.6 | |
| 1.23 | 0.048 | 0.168 | 1.44 | 5.32 | 28.7 | 24.1 | 44.3 | 51.1 | 11.9 | 9.7 | |
| 2.21 | 0.074 | 0.323 | 3.59 | 8.86 | 24.3 | 24.2 | 18.2 | 29.3 | 7.5 | 7.6 | |
| 1.81 | 0.057 | 0.265 | 3.15 | 7.68 | 18.9 | 16.4 | 14.6 | 25.4 | 6.0 | 4.6 | |
| 2.06 | 0.065 | 0.296 | 3.27 | 8.36 | 22.0 | 20.0 | 16.9 | 26.8 | 6.6 | 6.0 | |

starch was determined in triplicate because it was difficult to secure uniform samples from the coarsely ground materials.

From a review of the figures obtained it is apparent that the crude fiber results indicate more definitely the removal or the addition of bran than do any of the other determinations. The pentosan determination is next in this respect. The ratios of starch to crude fiber, starch to ash, and starch to pentosans are significant. For example, when 10 per cent of the bran was removed the maximum figure for crude fiber found was 1.62 per cent, whereas the minimum figure found for flour from the whole wheat berry was 2.23 per cent, a difference of 0.61 per cent. The pentosans show a difference of 0.66 per cent. With these same flours

the minimum and maximum starch to crude fiber ratios varied from 37.8 to 26.4, a difference of 11.4; starch to ash 48.2 to 35.9, or 12.3; and starch to pentosans 10.7 to 8.8, or 1.9. Thus there is a definite dividing line between the two flours.

While it is recognized that these data should be extended by analyzing more samples, including a wider variation in the bran content, it is believed the results given will serve as a guide in the interpretation of whole wheat flour analyses.

AN OPEN LETTER TO ALL SUGAR TECHNOLOGISTS.

At the Third Congress of the International Society of Sugar Cane Technologists, held at Sourabaya, Java, in June, 1929, the following resolution, in part, was unanimously adopted:

Be it resolved that a new periodical be started containing (or an existing periodical be requested to print) adequate abstracts in the English language, submitted by the authors themselves of all technical papers of more general importance.

The committee appointed in accordance with this resolution and consisting of H. P. Agee (Hawaii), chairman; K. Douwes Dekker (Java), R. Fernandez Garcia (Porto Rico), A. H. Rosenfeld (Louisiana), and W. B. Saladin (Cuba), has given this project very careful consideration and has discussed a number of plans. As a result the chairman of the committee, with the approval and consent of the other members, has authorized the general chairman of the Society to enter into negotiations with Mr. E. W. Mayo, Editor of Facts About Sugar, regarding publication of abstracts. The society is at present financially unable to employ paid abstractors, but it is believed, and this belief is expressed in the text of the resolution, that every author who is interested in having his work published and made known to the world is quite as much interested in securing a large circle of readers for his own publications, locally as well as universally, and also in having ready and convenient access to all the literature in his field appearing anywhere in the world.

A certain amount of editing of the abstracts is necessary, to be sure, in order to guarantee uniformity. This part of the work will be handled by the technical editor of Facts About Sugar, in cooperation with the officers of the Society.

The cooperation of all the sugar journals all over the world, of government departments, experiment stations, local technologists' associations, etc., will be necessary and is earnestly solicited. Reprints of the abstracts will be furnished at cost, in the more handy form of 15 x 23 cm. with two columns instead of three.

The success of the journal will depend entirely on the interest shown and on the amount of cooperation given by each member of the Society and by each author, whether or not he is a member of the Society.

The publication of abstracts will begin as soon as a sufficient number is received by the Editor of Facts About Sugar, 153 Waverly Place, New York, N. Y. Orders for subscriptions to the reprints should also be addressed to him. It is intended to include all articles which have appeared since January 1, 1930, so as to have a complete record for the calendar year. Authors please note.

The responsibility for the success of the new venture rests upon each individual sugar technologist, and those interested may obtain detailed information by addressing the General Chairman, 80 South Street, New York, N. Y.

F. W. ZERBAN, *General Chairman.*

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